STUDY OF CORNEAL EPITHELIAL THICKNESS IN DRY EYE USING ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY

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LIST OF ABBREVIATIONS

OSDI	Ocular surface disease index
T-BUT	Tear film breakup time
ST	Schirmer test
NS	Not Significant
AS	Anterior segment
OCT	Optical coherence tomography

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ABSTRACT

Aim- To study the corneal epithelial thickness in dry eye using anterior segment optical coherence tomography.

Methodology- It was a cross-sectional study that took place from November 2020 to April 2022, enrolling 52 patients with symptomatic dry eye and 52 healthy people as controls. A thorough inspection of the ocular surface was performed after completing the OSDI questionnaire. For each example inside the 0 to 9 mm zone, the average epithelial thickness was calculated.

Results- Clinical evaluation of ocular surface showed DED patients had significantly more symptoms (OSDI), lower TBUT and lower Schirmer I test score compared to control subjects. The DED group's mean corneal epithelial thickness was substantially higher. The central zone's thickness was likewise significantly different between the two groups.

Conclusion- Based on AS OCT results, it was determined that dry eye cases within the 0 to 9 mm zone had an increased average epithelial thickness.

INTRODUCTION

The cornea, which is transparent and covers the iris, pupil, and anterior chamber of the eye, is the front part of the eye. The cornea refracts light along with the anterior chamber and lens, making up around two-thirds of the eye's total optical power. The cornea's refractive power in humans is roughly 43 dioptres. The human cornea has five layers- corneal epithelium, bowman's layer, corneal stroma, descement's membrane and corneal endothelium.

The corneal epithelium keeps the cornea's optical quality intact. Its average refractive power across the middle 2.00mm diameter zone is 1.03 diopters (D). Therefore, changes in the thickness and distribution of the corneal epithelium may be one of the early warning indications of ectasia, dystrophy, and contact lens-associated keratopathy, among other corneal diseases. [1]

Dry eye disease (DED), a multifactorial disorder, has the ability to impair the conjunctival and corneal epithelium through influencing tears and the ocular surface. Some of the reasons of DED include the instability of the tear film, increased tear osmolarity, abnormalities in the meibomian and lacrimal glands, and a string of inflammatory processes in the epithelial surface cells. Clinical ocular symptoms of DED, such as eye pain, photosensitivity, and changing vision, could have been brought on by the damaged corneal epithelium. In addition to the severe symptoms and detrimental effects on quality of life, it may be extremely challenging to assess individuals for refractive surgery. The thickness of the corneal epithelium can be mapped to determine the morphological signs of epithelial deterioration. Several devices are used to measure the corneal epithelium thickness: in vivo Confocal Microscopy, very high frequency ultrasound and anterior segment optical coherent tomography (AS-OCT).

A recent study about dry eye disease from north India reported a prevalence of 32% and based on symptoms 81% were severe DED. Dry eye is becoming more common as a result of environmental causes and evolving lifestyles.[2]

DED can be effectively prevented through consolidated efforts of community, professional and individual actions. In most cases, early detection and intervention is crucial to improve the condition using therapeutic regimes. In the hunt for an objective, repeatable, and quantitative clinical test that may help in the differential diagnosis of dry eye, the notion of corneal epithelial thickness as a viable tool in dry eye evaluation can be effectively used. Using epithelial maps produced by optical coherence tomography, an important anatomical characteristic known as epithelial thickness has recently been examined in dry-eye patients. The kind and dosage of lubricant to treat dry eyes can be determined depending on the severity of the condition. Because it strongly correlates with symptoms, the epithelial thickness profile range may be used to track patients and how well they respond to treatment.

AIM AND OBJECTIVES

AIM

To study the corneal epithelial thickness in dry eye using anterior segment optical coherence tomography

OBJECTIVES

1. To assess the effectiveness of anterior segment OCT in the Vijayapura district for dry eye corneal epithelial thickness mapping

2. To assess corneal epithelial thickness mapping in normal eyes using anterior segment OCT in and around the Vijayapura district,

3. To correlate the patient's symptoms with the corneal epithelial thickness profile in both dry and healthy eyes.

REVIEW OF LITERATURE

EPIDEMIOLOGIC STUDIES IN DRY EYE.

- **The Beaver Dam Eye Study [3]**

  The Beaver Dam Eye Study (BDES) was a population- cohort study that consisted of 3,722 residents of Beaver Dam, Wis., and USA. The subjects ranged in age from 48 to 91 and were mostly Caucasians. On a five-year follow-up questionnaire, the prevalence of dry eye was self-reported, and it was 14.4%. In older age groups and among women, dry eye was more prevalent. Men had an age-adjusted prevalence of 11.4%, while women had one of 16.7%. Smoking and a history of arthritis were two additional risk factors for dry eyes. The subjects' consumption of caffeine was found to be protective.

- **The Salisbury Eye Study [4]**

  A population-based study called the Salisbury Eye Study was conducted to analyse the demographics and determine the prevalence of dry eye among older Americans. 2,520 participants between the ages of 65 and 84 who resided in Salisbury, Maryland, made up the study's sample. Using a six-item questionnaire, researchers evaluated the signs and symptoms of dry eye, and participants completed Schirmer's and rose bengal tests.

The questionnaire served as the main source for the definition of dry eye. Subjects were deemed to have dry eye if they complained of at least one symptom frequently or always and had abnormal test results (Schirmer's score 5mm, Rose-Bengal score>5). When a patient had aberrant test findings but no symptoms, dry eye was not assumed to be present. Based on participants reporting symptoms, 14.6% of people had dry eye. According to those who had symptoms and a low Schirmer's score, the prevalence of dry eye was 2.2%; for those who had symptoms and a high rose bengal score, it was 2%. No association of symptoms or signs was seen with age, sex

, or race.

- **The Melbourne visual impairment Project [5]**

A population-based research of Melbourne, Australia, inhabitants called the Melbourne Visual Impairment Project (VIP) was conducted to investigate age-related eye illness. There were 926 people in the research population, ranging in age from 40 to 97. Researchers used a questionnaire, the Schirmer's test, the time it took for the tear film to break up, rose bengal staining, and corneal staining to evaluate dry eye. Each technique of assessment had a different prevalence of dry eye: 10.8% with rose bengal >3, 16.3% with Schirmer's test 8mm, 8.6% with tear film breakdown time 8 sec, 1.5% with fluorescein staining > 1/3, 7.4% with two or more signs, and 5.5% with report of any severe symptom not related to hay fever. Women were more likely than men to report having dry eye symptoms but no visible symptoms. People who reported a history of arthritis and those who were older were more likely to experience two or more symptoms.

- **The Canadian Dry Eye Epidemiology study(CANDEES) [6]**

The purpose of the Canadian Dry Eye Epidemiology study (CANDEES) was to determine the prevalence of dry eyes in Canada. All Canadian optometry clinics received 30 copies of a questionnaire as part of the study. 13,517 (15.7%) of the 86,160 surveys that were initially mailed were returned. The individuals ranged in age from under the age of ten to over the age of eighty.

28.7% of respondents to the study claimed to experience dry eye symptoms. 90% of the participants in this study claimed to have mild symptoms, 7.6% to have moderate symptoms, and 1.6% to have severe symptoms. Females and people with a history of dry mouth and allergy-like symptoms of the lids were among the population groups with a greater prevalence of dry eye.

Horwath -Winter et al found that pathologic abnormalities and subjective symptoms due to dry eye were more in patients with Sjogren's syndrome and dry eye syndrome improved or stabilized with appropriate treatment.[7] Risk factors for dry eye include advanced age and female gender , arthritis , smoking, multivitamin use[6] and hormone replacement therapy.[5]

Tsubota et al reported that local ocular surface conditions such as dry eye can significantly affect the pattern of blinking and that the use of artificial tearsor spectacles with moist panels and moist inserts tended to normalize the patterns of blinking while exposure to wind made the blinking pattern abnormal.[8]

Moss et al in another study of the Beaver Dam Eye Study reported that incidence of dry eye was significantly associated with increasing age.[9]

In a study of women who had premature ovarian failure and age-matched controls, **Janine A., Smith M. et al.** discovered that the women who had premature ovarian failure were more likely to have ocular surface damage and symptoms of dry eye than the age-matched controls. This information offered more proof of the complex role that sex hormone plays in maintaining the integrity of the normal ocular surface. [10]

In a prospective cohort of 22,382 diabetic patients, **Igor Kaiserman et al**. compared the prevalence of keratoconjunctivitis sicca with that in the general population and discovered that, after adjusting for age and gender, a significantly higher proportion of diabetic patients (20.6%) received ocular lubrication than non-diabetic patients(13.8%). For both sexes and across all age categories, the disparity was notable. Poor glycemic management led to an increase in ocular lubricant intake (mean annual HbA1c levels). This effect was found to be independent of age, gender, and place of residence by multivariate analysis. [11]

Goto E et al studied functional visual acuity in dry eye patients , which was measured after sustained eye opening for 10-20 seconds, as a simulation of visual function of daily acts of gazing , which is defined as looking at an object with involuntary blink suppression. It was an interventional non-randomized comparative trial. Ordinary best corrected visual acuity and functional visual acuity was measured in non- Sjogren's syndrome and Sjogren's syndrome patients and in normal controls. This study shows that the visual function of dry eye patients becomes abnormal with ocular surface irregularity when the eye is kept open for 10-20 seconds. Dry eye is not only a simple disorder causing discomfort to patientswith deteriorated quality of life, but also causes impaired visual function in daily life.[12]

- **ANATOMY AND PHYSIOLOGY OF THE TEAR FILM [13,14]**

The tear film is a three layered structure composed of lipid, aqueous andmucin layers from anterior to posterior.

It is more appropriate to think that tear film is a two layered structure ; a thin lipid film floating on a large aqueous lake. The mucin layer more appropriately belongs to the corneal and conjunctival epithelium to which it is closely attached. Thickness estimates ranges between 7 and 40μm, the range being difficulty in visualisation of tear film for measurement. The film is thickest after a blink, measuring about 9μm. The thickness then decreases in a linear manner until at 30 seconds it has decreased to its minimal thickness of 4μm.

- **FUNCTIONS OF TEAR FILM**

1. Establishes and keeps up a smooth corneal refracting surface.
2. Keeps the corneal and conjunctival epithelial cells in a moist environment.
3. It has antibacterial qualities.
4. lubricates the lids.
5. It transfers oxygen from air to the cornea.

6. It dilutes and washes away noxious irritants

- **The lipid layer**[14,15]

The lipid layer was first postulated by wolff and subsequently described by McDonald.This is the most superficial layer of the tear film , 0.1μm in thickness. It is produced primarily by the meibomian glands. These are modified sweat glands present in the tarsal plate about 30-40 in upper lid and 20-30 in lower lid. At the mucocutaneous junction of the lid margin, they exude sebum. Triglycerides, wax esters, cholesterol esters, and hydrocarbons are all present in the secretions. [16]

The lipid layer consists polar and nonpolar lipids. The polar lipids are in contact with the aqueous phase of the tear film and provide structural stability to the tear film , while nonpolar lipids are at the air interface.[17,18] The melting point of the lipids is 19-32^{o} C , that ensures that it is always fluid on the ocular surface.

- **Functions of lipid layer**

1. Prevents spillover of the tears and contains tears within the palpebralopening.
2. Inhibits evaporation of tears, especially under conditions of low humidity and turbulent airflow.
3. Prevents damage to the lid margin skin by tears.
4. The smooth layer of lipid provides an excellent dioptric element forlight refraction into the eye and sharp retinal image formation.
5. Acts as a hydrophobic barrier and prevents the aqueous layer from getting contaminated with polar lipids that could rupture the tear film prematurely.

- **Aqueous layer**

The aqueous layer is about 6.5μm in thickness. It comprises about 60% of the tear film. It is secreted by the main lacrimal gland and accessory lacrimal glands of Krause and Wolfring. The layer is an aqueous solution with low viscosity that includes enzymes, proteins, and glycoproteins, as well as ions from inorganic salts, glucose, and urea. Additionally present are lysozyme, lactoferrin, tear-specific prealbumin, and secretory IgA. [16]

A majority of the lacrimal gland fluid enters the fornices superotemporally from the fornices, lacrimal gland fluid travels , even in the absence of blinking , into the marginal tear strips. The distribution of this fluid in the marginal strips to the preocular film depends on the blink.[19]

- **Functions of aqueous layer**
    1. It gives the epithelium oxygen.
    2. Washes away debris and noxious irritants.
    3. It prevents infection due to the presence of antibacterial substances likelysozyme and betalysin.

- **Mucin layer**

The mucin layer is produced by the conjunctival goblet cells. Holly and Lemp estimated that mucus layer is 0.002 – 0.005 μm thick. But recent studies indicate that it is considerably thicker about 30μm. It is made up of glycoproteins and mucopolysaccharides. Goblet cells are interspersed among the stratified squamous epithelial cells of the conjunctiva.[20] These are distributed singly or in clusters which are identified as mucous crypts.[15] Some goblet cells release their mucus into the crypts that rise to the ocular surface, whereas others secrete straight onto the ocular surface. [20]

Kersing has shown that goblet cell densities vary over the ocular surface with the highest density in the inferonasal quadrant. Kersing, in his study found goblet celldensities of 400/sq

mm and 1,599/sq mm on the interpalpebral bulbar and inferior palpebral conjunctiva respectively. Conjunctival goblet cells have typically been identified by alcian blue and periodic acid Schiff stains. These stain the mucus within the secretory granules butnot in the remainder of the cell. These do not identify the goblet cells that have recently secreted mucus.

- **Functions of the mucin layer** –

1. The mucin of the glycocalyx renders the whole of the ocular surface hydrophilic and allows even spreading of the aqueous layer over the eye .[21]
2. An adequate layer of mucin masks lipid molecules arriving at the corneal surface and thereby maintains its hydrophilic character.
3. An estimate of the effectiveness of the mucin layer can be made by measuring the tear film break up time or by performing a goblet cell count.

The composition of tear film is as follows [16]

Contents	**Concentration**
• Water	98.2%
ELECTROLYTES	
• Sodium	145mEq/L
• Potassium	20mEq/L
• Chloride	128mEq/L
• Bicarbonate	26mEq/L
• Calcium	2.11mEq/L
• Magnesium	Trace
• Zinc	Trace

METABOLITES

- Glucose3mg/dl

- Lactate 1-5mmol/L
- Pyruvate present
- Urea 7.20mg/dl
- Total proteins 0.6-2gm/100ml
- Prealbumin 1-2gm/L
- Lactoferrin trace
- IgA 14-24mg/100ml
- IgG 17mg/100ml
- IgE 250mg/100ml
- Glycoproteins present
- Mucopolysaccharides present

ENZYMES

- Lysozyme present
- LDH high levels
- Peroxidase 10^3U/L

LIPIDS

- Cholesterol 200mg%
- Glucose

The glucose concentration present in the tear film is too low to satisfy the needs ofcorneal epithelium. Corneal glucose is obtained from aqueous humour.

PROTEIN COMPONENTS –

These include tear specific prealbumin , β-lysin, lactoferrin , lysozyme , immunoglobulins , complement . These lower the surface tension of the tears, thus maintaining a continuous tear film over the cornea.

LYSOZYME

Lysozyme in human tears was first described by Fleming in 1922. [14] lysozyme(muramidase) destroys bacterial cell membranes . Tear lysozyme levels have been shown to be decreased in keratoconjunctivitis sicca , lupus erythematosus , trachoma and herpes simplex. The lysozyme test is the sicca syndrome test with the highest sensitivity.

LACTOFERRIN [14]

This is both a bacteriostatic and bacteriocidal iron binding protein that accounts for upto 25% of human tear proteins. The normal concentration of lactoferrin is 1.4mg/ml.

IMMUNOGLOBULINS AND COMPLEMENT

All the immunoglobulins are present in tears, but only IgA is present in significant quantities about 14-24mg/100ml. Yamamoto and Allansmith showed that the entire complement pathway is present in normal human tears.

The average tear flow rate in humans is about 1.2µL/minute and ranges from 0.5-2.2µL/minute. It is lowest during sleep and highest during emotional stimuli or fall ofirritants such as foreign bodies. In the total 7µL of tear film , 1µL is in the preocular tear film within the palpebral fissure , 2.9µL is located in the peripheral tear film strips, and approximately 4.5µL within the fornices.

PHYSICAL PROPERTIES OF TEAR FILM [16]

1. Thickness of tear film – average thickness varies from 4-8µm. However recent confocal microscopy has shown that tear film is about 40µm thick.
2. Volume of tear film – average volume of tear film is 7µl with a range from 4-13µl during basal conditions.
3. Rate of tear secretion – in non-stimulated subjects the average rate of tear secretionis 1.2µl/min, with a total 24 hour secreting volume of about 10cu ml.
4. Turnover rate – is 18%/min.

5. Refractive index – refractive index of tear film is about 1.357.
6. pH of tears – usual range is from 7.3 to 7.7.
7. Osmolarity of the normal human tear film averages 302+-6(SD)mOsm/L.
8. Oxygen tension - in the normal tear film under basal conditions, po_2 varies from40-160mm Hg.

BLINKING AND TEAR FILM STABILITY

Tears are produced normally at a rate of 1-2µl/minute and an average volume of 5 to 10µl is in the conjunctival cul-de-sac.[22]

The normal involuntary blink takes about one fourth of a second, and occurs an average once every 5 seconds. However, blink rate does reduce with activities that require concentration such as reading, driving and watching television. The human tear film is a constantly changing fluid membrane with flowoccurring only in the aqueous layer. The lipid layer remains intact between blinks. The mucin layer remains adherent to the epithelium.

DIAGNOSIS OF DRY EYE [23]

Symptoms form an important part of assessment of any disease process and dry eye is no exception . Surveys on population based prevalence of dry eye have shown that symptoms are present in 25-35% of people.[6] However , studies have also shown a poor association between the signs and symptoms of dry eye.[24] The full spectrum of symptoms include heaviness of the lids, blurring and fluctuating vision , excess ropy mucus, burning , itching , scratchiness , foreign body sensation , photophobia, tearing and pain. The symptom most frequently encountered is foreign body sensation or sandy sensation.

Often the patients volunteer information about their intolerance to drafts and winds , intolerance to air conditioning.

Patients with dry eyes frequently struggle to read since less blinking occurs when concentrating

on a task. As the blink frequency goes down, the length of the time the eye is left exposed to the atmosphere becomes longer and drying may increase.[23]

Patients may complain of night time awakening especially in case of blepharitis or lagophthalmos. Sleep decreases tear production and compromises the eye with regard to tear flow and produces nocturnal symptoms.

Since smoke is actually a suspension of solid in air, it bombards the ocular surface with tiny particles that are uncomfortable to people with tear-deficiency.

Asking about past skin conditions is vital when diagnosing conditions including scleroderma, scurvy, the face rash of lupus, ancient stevens-johnson syndrome scars, and acne rosacea.

History of drug intake is asked. Thiazide diuretics, antidepressants, β-blockers, anticholinergics, antihistaminics, anti-parnkinsonian drugs, benzodiazepines, antihypertensives are known to cause dry eye.

It is helpful to have a list of standardized questions to ask patients, using defined terms. Numerous studies have been done to find the most common symptom and to formulate a valid questionnaire.

The National Eye Institute visual function questionnaire (NEI VFQ – 25) is one such questionnaire to assess the symptoms of ocular disease . However , itsurveys the general ocular health and is not reliable to capture the broad rangeof symptoms unique to a certain ocular disorder.[25]

The ocular surface disease index questionnaire (OSDI), is a 12 item questionnaire designed to provide a rapid assessment of symptoms of ocular irritation consistent with dry eye disease and their impact on vision relatedfunctioning .The questions were generated based on patient comments from several years of clinical studies . Each symptom is given an individual score and the final calculation takes into account the number of questions answeredand the cumulative scores . The reliability and validity of this questionnaire has been tested

in a sample of 109 dry eye patients where it has been found to have excellent test-retest reliability and validity effectively discriminating between normal, mild to moderate, and severe dry eye disease as defined by both the physician's assessment of severity and a composite disease severity score.[26] Like it has been with other trials , OSDI too has shown to have moderate co-relation with clinical signs among patients with dry eye disease who have tear deficiency . But , it has demonstrated good sensitivity and specificity in distinguishing between normal subjects and patients with dry eye .

OSDI is scored on a scale of 0 to 100, with higher scores representing greater disability. Due to these reasons , this questionnaire has been employed inthe present study.

EXAMINATION

Non-ocular examination

General physical examination is undertaken to note any arthritic changes , facial skin changes, salivary gland enlargement . The mouth isexamined for evidence of xerostomia.

Ocular examination

One of the most remarkable features of early dry eye syndrome is that the eye appears to be perfectly normal .

Decreasesd visual acuity that varies with blinking is one of the first signs encountered[42.]

The initial slit lamp examination is to be done without any topical anaesthesia or special stains into the eye.

The configuration of the lid margin , its approximation to the ocular surface and the completeness of the voluntary lid closure are noted.

The palpebral fissure width is to be examined which is important in the understandingof dry eye because the tear film evaporation is proportional in part to the ocularsurface exposed. The presence of ectropion , entropion ,trichiasis, lid erythema , telangectasia, poliosis, loss of lashes, colarettes, foamy discharge or inspissated material from meibomian gland are noted.

The bulbar and palpebral conjunctiva are examined for dilated conjunctival vesselsand tenacious stings of mucus which are common in keratoconjunctivitis sicca. Redundant , thickened and loose superior bulbar conjunctiva is seen in superior limbic keratoconjunctivitis. Conjunctival subepithelial fibrosis , keratinisation, symblepharon, and vascularisation are often seen in cicatrizing disease such as ocular Cicatrial pemphigoid and Stevens-johnson syndrome.

Inferior marginal tear film strip-

The size of the inferior marginal tear strip is an indirect indication of tear film volume. The height of the marginal tear strip is measured between the upper margin of the lower lid and the globe. A normal strip is 1-2mm above the lid margin of the lower lid with a concave anterior surface. The slighty deficient strip less than 1mm above lid margin, an enlarged strip will have a convex surface and more than 2mm in size. The deficient inferior marginal strip appears absent with little evidence of tears in the juncture between the lower lid and the globe. The size of the inferior marginal strip is not an absolute sign of dry eye.

Pre-corneal tear film [14]

The pre-corneal tear film should be examined before manipulating the lidswith the slit lamp microscope. Mucus particles and debris floating up and down against a background of focal gray epithelial dots may be seen in the interpalpebral area suggestive of keratoconjunctivitis sicca. Small pieces of debris called meniscus floaters are transported between the upper and lower tear menisci. Patients with dry eyes experience them very frequently. Pre-corneal tear film mucus strands are actually rolls of lipid-contaminated mucus that have been forced into a cul-de-sac by the shearing action of the lids. In aqueous deficient conditions, these are typical, but in mucin deficient situations, they can be quite spectacular.

Cornea-

The epithelial abnormalities commonly found are dry spots and punctuate epithelial keratopathy which are best seen with the slit-lamp as gray dots localised over the inferior surface of the cornea.[22]

Cornea is also examined in the interpalpebral area to detect localised elevations and corneal thickness, which is reduced at the centre in patients with dry eye.Filaments are short(<2mm), discrete, translucent, bulbous strands of mucus intertwined with desquamated cells and cellular debris that dangle from the corneal surface and stain with rose bengal . These are characteristically located on the inferior one-third of the cornea . Blinking produces severe pain because the filaments are firmly attached to the richly innervated epithelium.

CLINICAL DIAGNOSTIC TESTS

Tests to assess tear function can be broadly classified as tests that measure tearsecretion , those that measure tear film stability and those that measure clearance.

TESTS OF TEAR SECRETION

- **Schirmer's tests**

This test is intended to provide a measure of tear production per unit time.

This was the most common technique for the assessment of tear secretion which was originally described in 1903.[23]

- **Schirmer's 1**

This can be done with or without topical anaesthesia which measure only basicand combined basic and reflex (total) secretion respectively.

It is performed by using No.41 Whatman filter paper that is 35mm long and 5mm wide. A

notch is present at 5mm from one end and indicates the position of the lidfold that will help hook paper onto the lower lid . It is placed at the junction of the middle and lateral 1/3 over the lower lid . The patient with the eyes open, in a dimly lighted room, looks straight and blinks normally. Both eyes are tested simultaneously. Care should be taken not to touch the cornea. After a full five minutes, the strip is removed and the wetted length is measured from the fold. Normal values without anaesthesia are >/ 10mm wetting /5 min.

Basic secretion is measured by anaesthetising the conjunctiva by 4% xylocaine. Normal values with anaesthesia are >/5mm wetting /5 min. The difference in the basicand total secretion gives the amount of reflex secretion.

Pathological values

Borderline dry eye - <10mm/5min

Hyposecretive dry eye -<5mm/5min

- **Schirmer's 2 -**

This is performed as in schirmer's 1, but after the filter is in place a dry cotton bud is placed in the nares to irritate the nasal mucosa. The rationale for this test is that the ocular surface receptors are fatigued due to the constant stimulation in a dry eye state and therefore the stimulus of the filter paper does not induce a reflex secretion.[23]

Stimulating the nasal mucosa irritates the trigeminal nerve and since this is another afferent stimulus for reflex tear secretion, results in tear production .this test is very uncomfortable for the patient. It involves vigorous stimulation of nasal mucosa. Wetting of the strip in response to this test is reduced in Sjogren's syndrome. Less than 15mm wetting indicates failure of reflex secretion.

- **Schirmer's 3**

This is seldom performed and involves the use of a strong photic stimulus to produce reflex tearing due to a retinal reflex.

Lucca et al., evaluated the sensitivity and specificity of the schirmer's test and found a 25% sensitivity and 90% specifity for this test using history , symptoms and clinicalexamination.[23] Although a diagnosis of dry eye cannot be made or dismissed on the basis of this test alone, it is one of the crucial diagnostic tests in the assessment of dry eye syndrome.

Hamano et al., developed the phenol red thread test in an attempt to overcome some of the disadvantages of schirmer's tests. 3mm of dye impregnated 15mm cotton thread is placed under the lateral $1/5^{th}$ of inferior palpebral lid margin. It is allowed to absorb tears for 15seconds. Its colour changes to bright orange from tear contact . asian population show a lessened wet length response . The Japanese diagnostic criteria uses a cut-off value of 10mm for the phenol redthread test.[27]

TEAR MENISCOMETRY

It gauges the traits of the tear meniscus. Reduced height and radius of curvature of the tear meniscus in aqueous deficient dry eye are indicators of decreased tear meniscus volume. To give a non-invasive approach of diagnosis, the radius of curvature can be evaluated using reflected meniscometry or slit image photography.

MEASUREMENT OF TEAR FILM STABILITY :

- **Tear film break up time (TBUT)** [22,23]

Tear film break up time was originally described in 1969 by Norn. Lemp and Hamill in 1973 popularised the concept. It is a practical method of assessing the stability of pre-ocular tear film.

A fluoroscein strip , moistened slighty with balanced salt solution or similar ocular irrigant is touched against the inferior tarsal conjunctiva and the patient is asked to blink several times to distribute the dye throughout the tear film. The examiner should encourage the patient to stare straight ahead without blinking, while the cornea is observed through the slit-lamp using

diffuse illumination with the cobalt blue filter. The time between the last blink and the appearance of the first randomly distributed dry spot in the fluroscein film is noted in seconds. Normal tear film break up time is greater than or equal to 10seconds. It is susceptible to several variables such as

1. Lid holding and use of topical anaesthesia which reduces the tear break-up time.
2. Environmental conditions such as humidity and air flow.

Tear break up time does not vary with age and gender.

The break up of the tear film should occur in a random pattern so that no one area consistently shows dry spots. An area that consistently breaks up indicates localised corneal surface irregularities. Tear film break up time is reduced in patients with mucin deficiency and in patients who have severe aqueous deficiency. The normal break up time varies from one individual to another and also may vary in the same individual at different times of the day.

Holly proposed that the pre-corneal tear film thins due to evaporation and retracts towards fornices with each blink. The mucin surface becomes a hydrophobic surface as the superficial lipid layer diffuses through the aqueous layer to it. The aqueous film then retracts from the contaminated areas , forming a dry spot . These appear usually with in 15-30seconds after a blink at scattered locations on the corneal surface.

In normal individuals, the blinking action of the eyelids which usually occurs before the formation of dry spots is required to reform the layer and hence, the blink interval should be shorter than the break up time .

- **Non invasive breakup time [23]**

This can be measured using a keratometer and a toposcope which was invented by Tonge in London.

- **Ocular surface staining**

Using vital and supravital stains, epithelial damage to the exposed surface of the eye can be shown.

- **Fluroscein** [27]

The most common technique for showing ocular surface injury is fluroscein staining. Increased epithelial permeability is indicated by this water-soluble dye staining the ocular surface after penetrating the intercellular gaps. This orange dye is applied to the eyes with a strip dipped in saltwater to make it flash green when aroused by blue light. Before applying, excess colour is brushed off the strip.

Staining typically only affects the exposed interpalpebral region of the ocular surface; but, in cases of extreme dry eye, it may also affect the upper bulbar conjunctiva.

Fluorescein instillation is very well tolerated and causes minimal irritation . Results are recorded on a corneal diagram. Before damaged cells are revealed by the stain, the precorneal tear film is coloured by the dye and it is possible to examine itfor normal variations in uniformity or for the appearance of dark patches when the layer breaks up clinically. Fluorescein differs from rose bengal because it stains areas of epithelial cell loss and not devitalised epithelium.[28]

- **Rose Bengal staining**

Rose Bengal is a water-soluble red aniline dye which is the tetraiodotetrachloro derivative of fluorescein.[14,22] When healthy epithelial cells are not shielded by a healthy layer of mucin, rose Bengal stains them, as demonstrated by **Fenestra and Tseng**. This offers the special ability to assess the preocular tear film's level of protection.

Rose Bengal does not stain the pre-ocular tear film as does fluorescein. It seems to precipitate at the bottom of the meniscus. It neither penetrates into the corneal stroma nor diffuses into the intercellular spaces of the epithelium like fluoroscein. It stains mucus particles, strands,

filaments and plaques more vividly thandoes fluorescein. It appears that staining depends on loss of cell surface glycoprotein that normally contribute to the glycocalyx and enable the mucous layer to attach to theocular surface.[19] Rose bengal is available as 1% ophthalmic solution and as dye impregnated strips.The strips are used by first applying unpreserved saline to it and then touching the wet strip to the inferior palpebral conjunctiva. Based on two variables—intensity and location—rose Bengal staining in dry eyes is to be interpreted.

The temporal bulbar conjunctiva, nasal bulbar conjunctiva, and cornea are the three zones that make up **Van Bijstervald's** scoring system for rose bengal dye. Each zone receives a score between 0 to 3, where 0 denotes no staining and 3 denotes confluent staining. Each eye's scores are added up, and any eye with a score of 4 or higher is said to have tested positively for sicca keratoconjunctivitis.

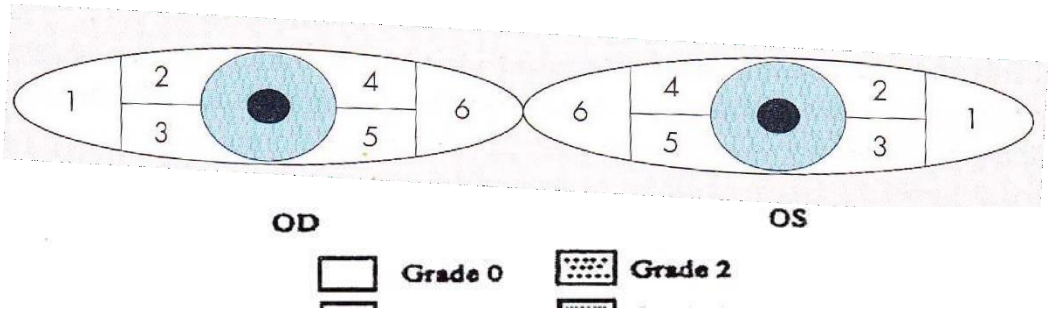


FIG 1. Modified Van bijstervald rose bengal grading map

Two false positives can be seen with rose bengal stain. A small amount of stain over the body of pterygium or pinguecula is a common normal finding . Also ifa schirmer's test has been performed before the use of a rose bengal stain , the conjunctiva will pick up the dye in that area of contact between the conjunctiva and paper strip . False negative results are seen in mild dry eye syndrome. In this system , normal eyes were accurately distinguished from abnormal eyes with 4% false positiveand 5% false negative results.

The interpalpebral conjunctiva, which appears as two triangles with bases at the limbus, is the typical site for rose bengal staining in aqueous tear insufficiency. The stainig pattern characteristic of dry eye should involve the exposure zone more than the non-exposure zone.

Patterns of staining in different dry eye conditions

- The conjunctiva stains more in keratoconjunctivitis sicca caused by lacrimal gland failure than the cornea. Staining in the early stages of the disease is only present in the nasal bulbar conjunctiva that is in the exposure zone. Within the exposure zones, nasal and temporal bulbar conjunctiva are stained in mild illness, with the nasal staining being more pronounced than the temporal. Later on in the illness, the inferior cornea begins to stain, and as it advances, the entire cornea becomes stained.
- Meibomitis and meibomian gland dysfunction-

  Rose bengal staining can initially be absent or present on the inferior or superior bulbar conjunctiva outside of the exposure zones and behind the eyelids. The staining spreads and impacts the cornea inside the exposure zone with more severe inflammation. Cornea stains more when compared to conjunctiva. The major disadvantage with rose bengal is its irritation. Care should be taken to flushthe eyes thoroughly after staining. Other stains used are lissamine green .

LABORATORY TESTS

- **Tear film osmolarity**

The gold standard test is the measurement of the osmolarity of the tear film. It is the most accurate and focused method of diagnosing dry eye. When 311mOsm/L is used as the upper limit of normal , elevated tear osmolarity is95% sensitive and 94% specific for dry eye.

- **Tear lysozyme:**

The lysozyme test has the highest sensitivity for sicca syndrome diagnosis. Meyer demonstrated that sicca keratoconjunctivitis resulted in a decrease in the tear lysozyme content as determined by viscometry. Regan verified what he had noticed. Thygeson and Kimura demonstrated that the lysozyme concentration dropped before any other sicca syndrome symptoms appeared. One of the common methods for measuring tear lysozyme is the method of agar diffusion which was introduced by **Van Bijstervald** in 1969. More recently , a radial immunodiffusion technique has been described. Tear lysozyme's "lack of specificity" as a dry eye diagnostic test is its biggest drawback.

- **Lactoferrin**

This is a protein produced by the lacrimal gland the level of whichcorrelates with tear volume. It has significant antibacterial activity. In the early 1980s, **Stutchell** and colleagues measured lactoferrin levels in dry eye patients and normal controls using electrophoresis and reported some interesting facts. Lactoferrin is measured using lactocard or lactoplate.[29]

- **Ocular ferning test**

The ocular ferning test is based on the observation that, when placed on a dry glass slide and examined under a microscope, conjunctival mucus from a normal eye crystallises into the shape of "ferns."

HISTOPATHOLOGICAL TESTS

To assess the conjunctival alterations that result from dry eye illness, impression cytology is utilised. This offers a qualitative way to assess changes in goblet cell density and conjunctival morphology at the light microscopic level. Impression cytology was introduced in ophthalmology by **Egbert** et al.,in 1977 and involves pressing and removing cellulose acetate paper on the ocular surface and staining the adherent cell layer.[26]

Before the introduction of this method, study of conjunctival surface wasattempted using excised pieces of tissue or by scraping the conjunctival epithelium. They experimented with a variety of methods including cellophane tape, photographic film, various synthetic filters and found that original Millipore filters were best for this purpose. It was introduced mainly to study the goblet cell density of the ocular surface in the initial period after introduction.

In the later years , the method gained popularity and became a standard method for study of ocular surface in various conditions such as viral conjunctivitis, keratoconjunctivitis sicca, vitamin A deficiency (where it can pickup mild xerophthalmia)[45] , changes occurring in conjunctival epithelium in chronic renal failure (where conjunctival epithelial features in chronic renal failure patients with or without calcium deposits have been studied), surface changes which may occur in psoriasis, ocular surface malignancies etc. [30]

However, the field of dry eye condition has seen the most application of this research. In order to study the histopathological changes occurring in the conjunctiva in dry eye states, Reddy M and colleagues conducted a comparative study of conjunctival biopsy versus impression cytology as early as 1991. They discovered that impression cytology was equally effective as biopsy for diagnostic purposes. It aids in determining the presence or absence of dry eye as well as the severity of dry eye. [31]

A recent study has shown that nylon paper can also be used as an alternative to cellulose

acetae filter paper for conjunctival impression cytology, with comparable results.[32]

Conjunctival impressions are obtained using Millipore cellulose acetate filter paper strips (3× 10mm size with a diagonal edge). After topical anaesthesia, with 4% xylocaine , a speculum is inserted and a blunt smooth edged forcepsis used to grasp the filter paper at one end and the paper applied on the temporal bulbar conjunctiva. The paper is softly pressed with a smooth glass rod. After two to three seconds, the paper strip is then peeled off. Additionally, the nasal portion has this repeated. The strips are dipped into a bottle of fixative, which is composed of 20:1:1 ethyl alcohol, formaldehyde, and glacial acetic acid. The strips are then stained with periodic acid Schiff and haematoxylin eosin and inspected under a microscope.

COMPLICATIONS :[33]

1. Sterile stromal ulcers : the corneal melt which occurs is typically an oval ,non infiltrated ulcers situated at or just below the visual axis with its longest dimension horizontal. The ulcer tends to progress quickly and then perforate .
2. Blepharitis and conjunctivitis : there is an increased incidence of infection dueto loss of normal antibacterial tear substances , lysozyme,β lactam and lactoferrin.
3. Band keratopathy
4. Keratinisation

5.Corneal vascularisation.

TREATMENT [34]

Ocular therapy for tear deficiency is directed towards the following goals:

1.Replacement using tear substitutes

2.Decreased tear drainage

3. Decreased tear evaporation

4.Improved surfacing by the tear film

5.Treatment of underlying disease

Tear substitutes

The goal of using tear substitutes is to increase humidity at the ocular surface and to improve lubrication with subsequent secondary benefits. Artificial tears smooth the corneal surface an effect that contributes to improved vision.

Dry eye tear substitutes contain 97% to 99% water. They also contain different electrolytes that are intended to keep the precorneal tear film's osmolarity between 303 and 310 mOsm/L and the nascent aqueous tear's approximate osmolarity of 300 mOsm/L constant, or to lower it without discomfort. Artificial tears have longer residence times when mucilages like methylcellulose are included.

Current therapy of dry eye disease is determined by the severity of the condition. In mild cases , in which there are no signs of damage to the conjunctiva or cornea, may be successfully managed with artificial tears appliedupto 4 times per day. In moderate cases, with mild damage to the corneaartificial tears can be used upto 12 times per day. In severe dry eye with features like keratinisation of conjunctiva, superficial punctuate keratopathy in addition to artificial tears and lubricating ointment , tear conserving therapies are required.

Artificial tears has obvious limitations. Firstly they cannot duplicate the composition of natural tears. Secondly the preservatives in them disrupt the precorneal tear film and damage the epithelial surface , worsening the ocular surface disease.The ideal tear substitute is that which approximates the normal electrolyte composition of the tear film , has low surface tension, is well tolerated , is non-irritating, contains no toxic preservatives, and has a long residence

time on the cornea and conjunctiva.

Preservation of tears

1. **Punctual occlusion** - helps in reducing the tear film osmolarity , increasing tear volume , and prolonging residence of artificial tears.

   Temporary occlusion – using collagen implants, silicone plugs . temporary occlusion can be done to ascertain if the punctal blockage will help reduce symptoms and also to rule out excessive tearing due to such blockage.

   Permanent occlusion – is done by using thermal or electric cauterization of puncta or canaliculi or by argon laser photocoagulation of the punctal opening.

2. **Moisture chambers and room humidifiers** - the concept behind moisture chambers is to enclose the eye so that evaporative loss of tears is reduced. Moisture chamber spectacles or goggles can be used.

Antiinflammatory therapy

Anti-inflammatory therapy should be considered for patients with severe keratoconjunctivitis sicca who have intolerable irritation, blurred vision or sight threatening corneal complications.

1. **Topical Cyclosporine A (CsA)**

Cyclosporine's immunosuppressive properties are related to the binding of particular nuclear proteins needed for the start of T-cell activation, which prevents T-cell synthesis of inflammatory cytokines like IL-2 and interferes with immune-mediated activities. Two objective indications of dry eye indicated a considerable improvement in patients receiving cyclosporine A 0.05%, 1%. (corneal fluroscein staining and schirmer value).

2.Topical Corticosteroids

Corticosteroids are strong inflammatory pathway inhibitors. They limit the production of inflammatory cytokines and chemokines, lower the level of matrix metalloproteins, lower the

expression of cell adhesion molecules, and increase lymphocyte apoptosis, among their many other biological functions. In numerous clinical investigations, they have been shown to relieve both dry eye symptoms and indicators. Unfortunately, corticosteroids cannot be used to treat dry eye over the long term due to their adverse side effects, which include cataract and steroid responsive glaucoma.

3. Oral tetracycline

Indicated in patients with meibomianitis. The mechanism of tetracycline action could be by inhibition of bacterial lipases. This inhibits the breakdown of meibomian lipids into potentially inflammatory fatty acids. Tetracycline 250mg orally 4 times a day , tapered over 3 months or doxycycline 100mg twice a day for upto 2 months before tapering to a maintenance dose of 100mg a day as long as needed.

4. Hot Compresses

Indicated in patients with meibomian gland dysfunction . A clean washcloth heated with hot water is applied to closed lids for 2 to 10 minutes. The warm compresses is followed by eyelid massage to express the secretions. Cleaning of the eyelid margins with dilute soaps such as baby shampoo may help in cases with seborrhoea.

Surgical treatment

1.Tarsorrhaphy

It can substantially reduce the exposed surface area of the cornea , thus reducing evaporation of tears. In patients with severe ocular surface disease , particularly persistent epithelial defects, and non-infectious corneal ulcers it can be extremely helpful.

2. Conjunctival transplantation

Cicatrizing ocular surface disorders associated with symblepharon , trichiasis, or cicatrising

lagophthalmos can be treated by conjunctival or limbal grafting and mucuous membrane transplantation.

3. **Keratoprosthesis**

Corneal prosthesis has been employed in the management of severetypes cicatricial disease such as ocular cicatricial pemphegoid , stevens-johnson syndrome, severe trachoma, and chemical burns in which scarring has been excessive and the prognosis for corneal grafting very poor.

- **Course of the disease**

Dry eye disease is a chronic disease, the treatment of which necessarily involves the patient compliance to a major extent. The patients of dry eye syndrome may remain symptomatic over the years with littleprogression of the disease.

Patients may go through periods of helplessness and depression due to the chronic nature of the problem which the ophthalmologist must recognise and encourage their patients to continue to pursue their normal activities and living.

EFFECTS OF DRY EYE ON CORNEAL EPITHELIAL THICKNESS

In 2007, Bela et al., carried out an investigation into the morphological and quantitative corneal properties in dry eye with various underlying pathologies. Dry eye patients showed significant alterations in the cornea, presumably due to increased desquamation of the superficial cell layer. This was most pronounced at the lower periphery of the cornea in patients with exposure keratopathy.[35]

According to **Kanellopoulos AJ and Asimellis G**'s study from 2014, women in their mid-forties had corneal epithelium that is statistically significantly thicker than that of an age-

matched control sample. In the questionable situations, increased epithelial thickness may be used as a clinically-validated, objective biomarker of dry eye. [36]

Fourier-domain OCT was employed by **Xinhan Cui et al.** in **2014** to show that the superior portion of the thickness map of the dry eye corneal epithelium was thinner than that of normal eyes. The superior and minimal epithelium was substantially thinner and had a wider range of map standard deviation in patients with more severe dry eye illness. [37]

The link between corneal and conjunctival epithelium thickness and ocular surface clinical tests in dry eye illness was examined by **Liang et al. in 2016**. They came to the conclusion that the mean CET in the experimental and control groups did not differ significantly. A thinner limbal epithelium and a thicker bulbar conjunctival epithelium were seen in dry eye patients, nevertheless. The severity of the symptoms of dry eye and the alterations to the tear film were associated to these changes. [38]

Nauman Hashmani et al., in **2018** conducted a study on Wide Corneal Epithelial Mapping sing an Optical Coherence Tomography and concluded that , a wide map can help improve the diagnostic accuracy in diseases of the peripheral cornea.[39]

In 2019**, Ma, Jack X. BA et al.** came to the conclusion that optical coherence tomography produced excellent repeatability and reproducibility for corneal ET and CT measurements up to a 9-mm zone in normal eyes and eyes with different corneal conditions. The study evaluated the repeatability and reproducibility of corneal epithelial thickness mapping for a 9-mm zone using this technology. [40]

In their paper Repeatability and Reproducibility of Corneal Epithelial Thickness Mapping with Spectral Domain Optical Coherence Tomography in Normal and Diseased Cornea Eyes published in 2019, Ruthi Sella et al. came to the following conclusion. In both healthy and sick

corneal eyes, the iVue SD-OCT offers good corneal ETM repeatability and reproducibility over all map zones. [41]

The use of corneal epithelial profile maps produced by an ultrahigh-resolution optical coherence tomography (UHR-OCT) in the diagnosis and treatment of dry eye illness was described and assessed by **Mohamed Abou Shousha et al** in their work published in 2020. (DED). They came to the conclusion that DED patients have irregular epithelium, an irregular epithelial thickness profile range, and an irregular epithelial integrity factor (EIF) that precisely correlates with patients' symptoms and can be used to monitor patients' responses to treatment. [42]

When comparing the corneal epithelial thickness profiles of dry eye patients, keratoconus suspects, and healthy eyes in 2020, **Susan Amana Ratan and Didar Anwar** came to the conclusion that the epithelium appeared to be thicker inferiorly in dry eyes and thinner in KC suspects. The displacement of the epithelial map's thinnest region could be a useful early indicator of keratoconus. To confirm that the narrowest location displacement assisted in this diagnosis, however, further research is required. [43]

In order to assess the central corneal thickness (CCT) and central corneal epithelial thickness (CCET) in people with Type 2 diabetes mellitus, **Elif Eraslan et al.** did a study in 2022. They discovered that the CCET was statistically narrower whereas the mean CCT was thicker. Because advanced diabetic retinopathy patients have thinner corneal epithelium, corneal epithelial diseases are more common in these patients. [44]

MATERIALS AND METHODS

The study was conducted in the department of Ophthalmology of B.L.D.E. (D.U.) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura. The study was conducted after obtaining clearance from institutional ethical committee of B.L.D.E. (D.U.) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura.

52 patients with symptoms dry eye and 52 healthy controls were included in this cross-sectional investigation.

STUDY DESIGN: Cross-Sectional Study

SOURCE OF DATA: Patients attending out patient department in B.L.D.E. (D.U.) Shri. B. M. Patil Medical College, Hospital and Research Centre in Ophthalmology department.

DURATION OF STUDY-18 months (November 2020 to April 2022)

SAMPLING.

The standard normal deviate for $\alpha = Z_\alpha = 3.2906$

The standard normal deviate for $\beta = Z_\beta = 3.0903$

$C = 0.5 * \ln[(1+r)/(1-r)] = 0.9076$

Sample size

With Anticipated correlation between and TBUT and OSDI Score in dry eye r= -0.720 (ref), at >99%confidence level and >90 power in the study, the sample size worked out is 52

Formula used is

$$N=\left[\left(\frac{Z_\alpha+Z_\beta}{C}\right)\right]^2+3$$

The standard normal deviate for $\alpha = Z_\alpha = 0.001$

The standard normal deviate for $\beta = Z_\beta = 0.001$

$C=0.5*\ln[\frac{1+r}{1-r}]=0.9076$

N=52

Sample size in control group=52 (assuming 1:1 group sizes)

Total sample size=52+52=104

INCLUSION CRITERIA

1. Patients who visited ophthalmology OPD and gave their consent to participate in study
2. Conditions causing dry eye such as steven johnson syndrome, sjogren syndrome, rheumatoid arthritis and collagen vascular disease.

EXCLUSION CRITERIA

1. Patients who have recently received artificial tear drops or who have worn contact lenses in the past
2. Patients with history of foreign body on ocular surface, Glaucoma, Ocular trauma or ocular surgeries

METHOD OF COLLECTION OF DATA:

The study was conducted in the department of Ophthalmology of B.L.D.E. (D.U.) Shri B.M.Patil Medical College, Hospital and Research Centre, Vijayapura. —In this cross-sectional study, 52 symptomatic dry eye patients and 52 normal subjects were enrolled. The subjects were selected from patients attending out patient department in Department of Ophthalmology, B.L.D.E. (D.U.) Shri. B. M. Patil Medical College, Hospital and Research Centre.

The participation in the survey was totally voluntary. At the time of the initial clinical appointment, each subject provided written informed consent for imaging and a dry eye exam. Each patient had a standard proforma filled out for them after being informed about the study and having their history, clinical findings, and investigations recorded. Additionally noted were prior treatment histories, pre-existing ocular conditions, and relevant local and systemic findings. Participants were then made to answer the ocular surface disease index (OSDI) questionnaire.

OSDI is generally a questionnaire containing 12 questions divided into three groups: symptoms of the eye (five questions), vision-related functions (four questions), and environmental factors (three questions). The threshold for dry eye symptoms was set at an OSDI score of 20 or above. The lead ophthalmologist was incharge of explaining the questions to patients and then recording their responses in order to limit any bias. On a scale from 0 to 4, the OSDI questionnaire is graded, with 0 denoting never, 1, some of the time, 2, half of the time, 3, most of the time, and 4, always.

All participants got a thorough evaluation of the ocular surface after completing the OSDI questionnaire, which was followed by the best-corrected visual acuity test, OCT scanning, TBUT, and Schirmer 1 test. TBUT was measured by instilling fluorescein into the inferior cul-de-sac and calculating the average of three consecutive break-up times. In a non-anesthetized eye, a 2% fluoroscein strip was moistened and inserted in the lateral third of the lower lid. The patient was instructed to blink just once or twice to prevent fluorescein from pooling, and then the strip was withdrawn. The time between the last blink and the first randomly distributed black discontinuity in the fluoroscein-stained tear film under the cobalt blue light of a slit lamp is used to calculate the tear break up time. Less than 10 seconds was regarded as abnormal. The lower lid's lateral third was covered with a conventional Schirmer's strip after a drop of proparacaine 0.5% was injected. The amount of strip wetness (measured in millimetres) was

reported after five minutes. Reading less than ten millimetres wetting was considered as positive Schirmer's test.

Patients who met the following criteria were classified as being in the control group:

(1) ocular surface disease index (OSDI) scoring less than 20,

(2) tear breakup time (TBUT) more than or equal to 10 seconds with ocular surface staining,

(3) Schirmer 1 test without topical anaesthesia (S1t) value higher than or equal to 10 mm/5 minutes

(4) There were no other ocular surface abnormalities visible with a slit lamp.

The DED diagnosis was as follows:

(1) The presence of dry eye symptoms (OSDI score 20)

(2) There is a qualitative or quantitative disturbance of the tear film (TBUT 5 seconds, or S1t 5 mm/5 min).

The entire corneal epithelium was topographically mapped using a novel anterior segment optical coherence tomography (AS-OCT) technique. Topographic epithelial thickness variations as well as average, centre, and periphery epithelial thicknesses were measured. Calculations were made to correlate epithelial thickness with dry eye symptoms.

IMAGING INSTRUMENTATION: The investigation used the ZEISS CIRRHUS 500 Spectral domain AS-OCT equipment with software version 8. Maps of the 9 mm-diameter cornea's total and epithelial corneal thickness were included in the data output. L-Cam lens, 8 meridional B-scans per acquisition, each with 1024 A-scans, and an axial resolution of 5 mm were the settings. After proper fixation and centering, the acquisition time per scan was in the range of a few seconds. Each case involved four separate acquisitions on the same day. The

same researcher took all measurements before doing the Schirmer lacrimation test and measuring the tear-film breakup time.

We measured, statistically analysed, and classified into zones the corneal thickness for each eye, ranging from 0 to 9 millimetres. Corneal epithelium thickness (CET) was defined as the epithelium thickness in the 2 mm central zone of the cornea; 2-5 mm in zone 1, 5-7 mm in zone 2, 7-9 mm in zone 3. The average, epithelial thickness was computed for each case within the 0 to 9 mm zone.

1. **STATISTICAL ANALYSIS:** To look for potential relationships between epithelial thickness, linear regression analysis was conducted. Receiver operating characteristic (ROC) curve analysis, comparative statistics, linear regression analysis, and descriptive statistics (mean, median, range, and standard deviation) were all performed using statistical software.

2. **THEORETICAL CONCEPTS AND EQUATIONS**

Concept of p value: The p-value is determined using the sample data, the test type, and the sampling distribution of the test statistic under the null hypothesis.

WHAT IS P-VALUE?

The p-value in statistics is the likelihood of experiencing results that are as extreme as those of a statistical hypothesis test, presuming that the null hypothesis is true. The p-value, which is employed as an alternative to rejection points, gives the minimal level of significance at which the null hypothesis would be rejected. When the p-value is smaller, there is stronger evidence in favour of the alternative hypothesis.

HOW IS P-VALUE CALCULATED?

P-values are computed using spreadsheets, statistical software, or p-value tables. A reader

could occasionally find it challenging to compare the outcomes of two distinct tests since different researchers employ various levels of significance when studying an issue. P-values offer an answer to this issue.

To get around this issue, the researchers might provide the reader with the p-value of the hypothesis test and let them determine the statistical significance. A p-value method to hypothesis testing is what it is.

P Value	Conclusion	Level of Significance
0.001 to 0.010	Reject Null hypothesis at 1% level	Highly significant
0.011 to 0.050	Reject Null hypothesis at 5% level	Significant
0.051 to 1.00	Accept Null hypothesis at 5% level	Not Significant

Table 1: Concept of P value

Following tests are used in this study for statistical analysis:

1. **Shapiro wilk test**

The Shapiro-Wilk test examines whether a sample of size x1,..., xn originated from a population with a regularly distributed population. The test statistic is

$$W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n}\left(x_i - \overline{x}\right)^2},$$

2. **Levene's test**

The dependent variable for Levene's test is the absolute value of the difference between a score and the mean of the group to which the score belongs. This test is equal to a 1-way between-

groups analysis of variance (ANOVA). W, the test statistic, is equal to F.

$$W = \frac{(N-k)}{(k-1)} \cdot \frac{\sum_{i=1}^{k} N_i (Z_{i\cdot} - Z_{\cdot\cdot})^2}{\sum_{i=1}^{k} \sum_{j=1}^{N_i} (Z_{ij} - Z_{i\cdot})^2},$$

3. **Mann–Whitney U test** -In statistics, the Mann–Whitney U test (also called the Mann Whitney Wilcoxon (MWW), Wilcoxon rank sum test, or Wilcoxon–Mann–Whitney test) is a nonparametric test of the null hypothesis that, for randomly selected values X and Y from two populations, the probability of X being greater than Y is equal to the probability of Y being greater than X

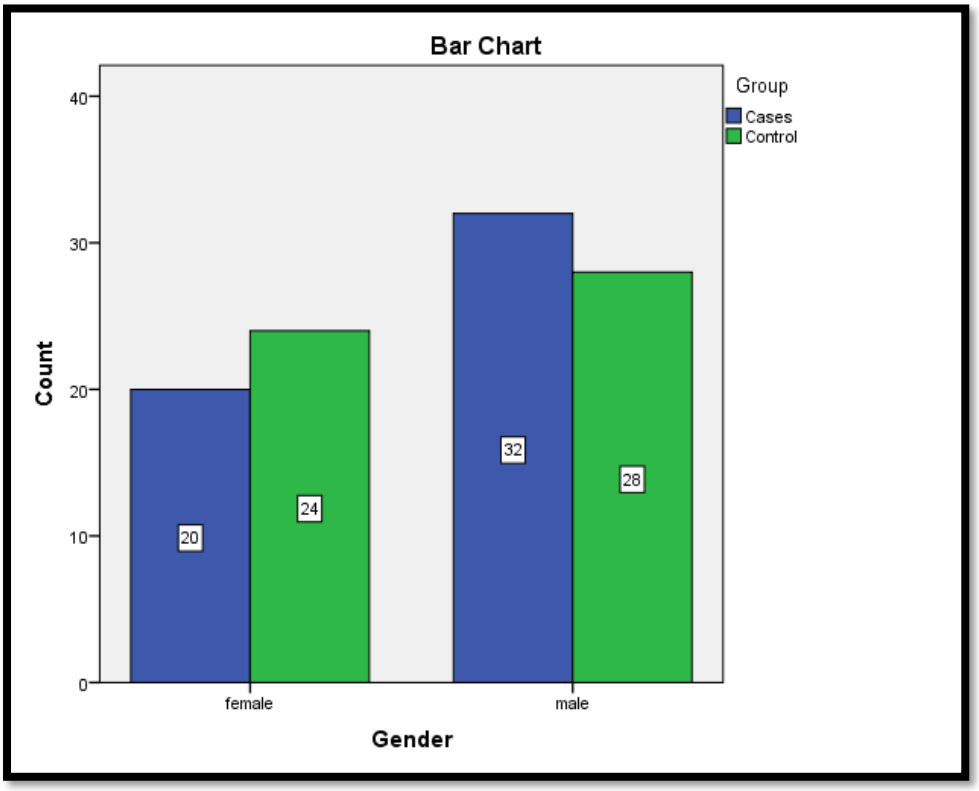
RESULTS

52 patients with symptoms dry eye and 52 healthy controls are involved in this cross-sectional study.

1. AGE DISTRIBUTION

In this study, adult patients from the age group 18 to 70 years were included.

2. SEX DISTRIBUTION



Graph 1. Bar graph showing Sex Distribution

			GROUP		TOTAL
			Case	Control	
GENDER	Female	count	20	24	44
		% within Group	38.5%	46.2%	42.3%
	Male				
		count	32	28	60
		% within Group	61.5%	53.8%	57.7%
TOTAL		count	52	52	104
		% within Group	100.0%	100.0%	100.0%

Table 2: Gender Group Crosstabulation

In the study 44 female were enrolled out of which 20 were categorized as case while 24 females served as control. Similarly, 60 males were enrolled out of which 32 were case and 28 were categorized as control. An increased percentage of males were found to be affected with dry eye.

Variables	Group	N	Mean	SD	Mann-Whitney U Test Value	P Value
Age	Case	52	42.808	14.087	1384	0.835
	Control	52	41.462	13.141		

Table 3: Age comparison for case and control group

The mean ages of the dry eye group and the control group were, respectively, 42.80 ±14.08 and 41.46±13.14. Regarding the age, there was no statistically significant difference between the groups (p-value: 0.835).

Table 4: Independent Samples- Mann-Whitney U test

Variables	Group	N	Mean	SD	Mann-Whitney U test value	p-value
Schirmer average	Cases	52	4.038	1.309	0.000	< .001
	Control	52	12.067	1.325		
T BUT average	Cases	52	4.058	0.662	0.000	< .001
	Control	52	16.673	3.082		
Central zone	Cases	52	56.077	4.044	2640	< .001
	Control	52	48.625	1.63		
zone 1 average	Cases	52	55.644	3.361	2627.5	< .001
	Control	52	48.288	2.175		
Zone 2 average	Cases	52	52.481	3.32	2393.5	< .001
	Control	52	48.346	1.638		
Zone 3 average	Cases	52	58.442	3.922	2690	< .001
	Control	52	48.413	1.927		
OSDI	Cases	52	29.596	6.2	2704	< .001
	Control	52	12.519	3.791		

The mean Schirmer test value for the dry eye group was 4.03±1.30 mm wetted on the paper after 5 minutes, whereas the mean value for the control group was 12.06 ±1.32mm with a p value less than 0.001.

For the dry eye group, the tear-film breakup time was 4.05±0.6 seconds while mean T-BUT value for control group was found to be 16.67±3.08 seconds. **(p<0.001)**

Dry eyes had significantly more corneal epithelium in all three zones as well as a thicker central corneal epithelium than normal eyes.

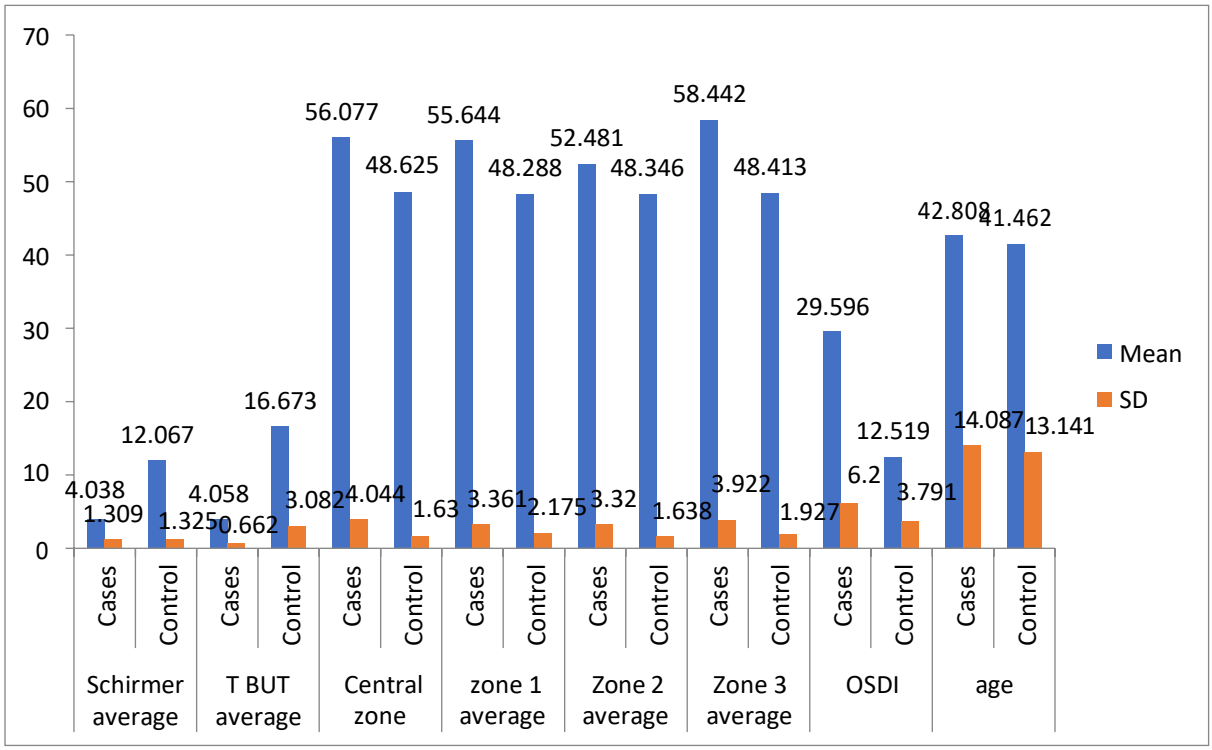
The central epithelial thickness was 56.07± 4.04µ for the dry eye group, compared to 48.62±1.63µ for the control group. (p<0.001)

Average corneal epithelial thickness for zone 1, zone 2, zone 3 was found to be 55.64±3.36 µ, 52.48±3.32 µ and 58.44±3.92 µ respectively in dry eye group. **(p<0.001)**

Average corneal epithelial thickness for zone 1, zone 2, zone 3 was found to be 48.28±2.17 µ, 48.34±1.63 µ and 48.41±1.92 µ respectively in control group. **(p<0.001)**

Therefore, there was a statistically significant difference between the control and dry eye groups in all pairings of the epithelial thickness measurements.

In comparison to the control group, the mean OSDI score for the dry eye group was considerably higher. (29.59±6.2 Vs 12.51±3.79)



Graph 2: Bar graph comparing various parameters between dry eye and control group

DISCUSSION

Because DED compromises the protective role of tears, the ocular surface is degraded. This results in the DED symptom spectrum, which may eventually lead to the loss of eye integrity. Techniques for diagnosing DED may rely on spotting the aberrant tear film, like in the TBUT or Schirmer's test. Poor correlation between such tests and patients' complaints has been found in studies [45, 46].

Since they are affected by factors that are challenging to control, such as fluctuations in dye concentration and light levels in surface-staining procedures, a lack of grading uniformity, etc., many of those tests lack adequate standardisation. Among other methods, TBUT and fluorescein staining scores have shown poor reproducibility and a significant operator dependence.

Ocular surface staining and the Schirmer's test are intrusive and uncomfortable for the patient. DED is a multifactorial disease that is hard to effectively regulate, standardise, or quantify since it is affected by several characteristics. A step-by-step strategy that focuses on the injury and effect that it causes may be effective. Recent innovations in imaging technology include confocal microscopy and OCT.

Confocal microscopy can diagnose DED, but it is a labor-intensive method that can only capture images at a small percentage of the total cornea and frequently necessitates contact with the ocular surface. The ocular surface can be imaged with OCT in vivo in a precise and secure manner.

The recently developed full-cornea corneal epithelial thickness imaging by AS-OCT may provide a useful clinical tool for qualitative (examination of 3-dimensional epithelial thickness mapping produced by interpolating successive meridional scans) and quantitative epithelium

evaluation due to the simplicity of noncontact application and speed of optical imaging (absolute average, central, and peripheral epithelium thickness measurements). [47]

Previous research has shown that the central corneal epithelial thickness of normal eyes ranges from 48.0 ± 5 to 59.9 ± 5.9 μm. [48-50]

Various techniques of data collecting employing the same technology, such as very high frequency ultrasound (VHFUS) versus AS OCT, may be to blame for the very broad range in central epithelial thickness reported in various papers (e.g., manual versus automatic measurements by using AS OCT).

The results of the current investigation revealed that the epithelium of dry eye patients was generally thicker and that there was a statistically significant difference in epithelial thickness between the case and control groups. The central thickness of the eyes with dry eyes and eyes with normal eyes varied by 7.45. This result was consistent with the information that **Qingfeng et al. and El Sanharawi et al.** had previously reported. [38,51]

In a mouse model of dry eye, **Fabiani et al. [52]** found that the average CET thickened significantly more in dry eye mice than in control mice. These results demonstrated that epithelial development and inflammatory processes had a considerable impact on the average CET during the early stages of DED. According to studies by **Chen et al.** [53] and **Kanellopoulos and Asimellis** [36], increased epithelial thickness may be used as an objective clinical indicator of dry eye.

The cell morphology of the epithelium associated with dry eye, which may include epithelial hypertrophy/hyperplasia, swelling cells, and an increase in the number of cellular layers, may be the cause of the increased thickness.

The strength of the current investigation is the AS-OCT screening method, which offers a

highly repeatable, quantitative, accurate, and straightforward to document approach. The measurement's benefits also include speed, the absence of corneal contact, ease of use, and reproducibility.

The CET in the dry eye group did not statistically vary from the CET in the control group. On the other hand, some researchers found contradictory results when it comes to CET in dry eye [37,54], while others found no differences between CET and control. The thinning of the corneal epithelium may be explained by the demise of stem cells near the limbus. The severity and length of the dry eye condition, the patients' ages, and the epithelial thickness measurement tools utilised may be to blame for the variance in CET results in dry eyes.

CONCLUSION

Dry eye is an underdiagnosed ocular condition due to the difficulty in diagnosing and evaluating dry eye due to the wide variety in illness symptoms and indications and the dearth of conclusive diagnostic testing. An suitable and standard questionnaire for dry eye examination, together with appropriate standard testing for dry eye, aid in diagnosis and therapy. Ocular comfort and satisfaction will be increased with early and effective management, improving overall quality of life. Based on the findings of the study, the following conclusions can be drawn:

- Imaging of the epithelium profile using ultrahigh resolution OCT and extrapolation of epithelial thickness variance can be used to diagnose dry eye disease, determine its severity, and track treated patients.
- A sign of dry eye is an increase in corneal epithelial thickness.
- The ability to customise contact lens designs for oxygen permeation and to suggest the right incision direction for dry eye patients undergoing refractive surgery should be made possible with knowledge of the epithelial thickness profile in these individuals.

SUMMARY

- OSDI was found to be an accurate indicator of dry eye symptoms. Higher OSDI scores, which indicate more severe dry eye, corresponded favourably with dry eye diagnostic tests.
- Schirmer test showed significantly decreased values in dry eye patients when compared to control group.
- TBUT was significantly decreased in cases with dry eye disease.
- Corneal epithelial thickness was found to be increased in dry eye group in all zones.

LIMITATIONS OF THE STUDY

- Limitations of our study were that the patients lacked the follow ups.
- The approach used in this study was cross-sectional, and there was no gender-specific stratification of epithelial thickness.
- Additional research with bigger sample sizes is required to support our findings.
- In the current investigation, the reproducibility of readings was not assessed.

BIBLIOGRAPHY

1. Simon G, Ren Q, Kervick GN, et al. Optics of the corneal epithelium. Refract Corneal Surg 1993; 9(1): 42–50.
2. Tityal JS, Falera C, Kaur M, Sharma M, Sharma N. Prevalence and risk factors of dry eye disease in north India: Ocular surface disease index-based cross-sectional hospital study Indian J Ophthalmol. 2018;66:207–11
3. Moss SE, Klein R, Klein BE . Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol 2000; 118 : 1264-68.
4. Schein OD, Munoz B, Tielsch JM, Bandeen-Roche K, W Sheila. Prevalence of dry eye among the elderly. American Journal of Ophthalmology 1997; 124:723- 728.
5. McCarty CA, Bansal AK, Livingston PM , et al . The epidemiology of dry eye in Melbourne, Australia. Ophthalmology 1998; 105:1114-9.
6. Doughty MJ, Fonn D, Richter D. A patient questionaaire approach to estimating the prevalence of dry eye symptoms in patients presenting to optometric practicesacross Canada. Optom Vis Sci 1997 ;74:624-631.
7. Horwath – Winter, Jutta , Berghold, Andrea, Schmut , Otto et al. Evaluation of the clinical course of dry eye syndrome. Arch Opthalmol 2003; 121: 1364- 1368.
8. Tsubota K, Hata S, Okusaua Y, Egami F, Ohtsuki T, Nakamori K. Qualitative Videographic Analysis of blinking in normal subjects and patients with dry eye. Arch Ophthalmol 1996;114: 715-720.
9. Moss SE, Klein R, Klein BE.Incidence of dry eye in an older population . Arch Ophthalmol 2004;122:369-373.
10. Janine A, Smith et al. Dry eye signs and symptoms in women with premature ovarian failure . Arch Ophthalmol 2004;122:151-156.
11. Igor Kaiserman et al. Dry eye in diabetic patients. Am J Ophthalmol 2005;139:498-503.

12. Goto E, Yagi Y, Matsumoto Y, Tsubota K. Impaired Functional visual acuity ofdry eye patients. Am J Ophthalmol 2002;133:181-86.

13. Lemp MA, Woifiey DE :The lacrimal apparatus. In : Hart WMJr , editors. Adler's physiology of the eye, clinical application. 9th ed. United States ofAmerica :Mosby year book, 1992 : 18-29.

14. Lamberts DW . Phsiology of the tear film. In : Smolin G, Thoft RA, editors. The Cornea Scientific foundations and clinical course. USA : Little , Brown and Company , 1994 : 439-485.

15. Dart DA, Sullivan DA. Wetting of the ocular surface. In : Albert DM , Jakobiec FA, editors. Principles and practice of ophthalmology. 2nd ed. Philadelphia : Saunders, 2000 :960-981.

16. Khurana AK, Khurana I. Anatomy and physiology of the eye. 2nd edition . New Delhi: CBS publishers and distributors ; 2006.p. 366-396.

17. Brown SI, Dervichian DG. The oils of the meibomian glands. Physical and Surface characteristics. Archives of ophthalmology 1969;82: 537-40.

18. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. Optometry and vision science 1997;74:8-13.

19. Gilbard JP . Dry-Eye Disorders . In : Albert DM , Jakobiec FA, editors. Principles and practice of ophthalmology vol 2 . 2nd ed. Philadelphia : Saunders, 2000 :982-1001.

20. Nelson JD, Wright JC. Conjunctival goblet cell densities in ocular surface disease Arch Ophthalmol 1984 July ;102:1049-1051.

21. Rolando , Zierhut. The ocular surface and tear film and their dysfunction in dryeye disease. Survey of Ophthalmology 2001 Mar ;45suppl 2:203-210.

22. Stulting RD, Mader TH, Waring III GO. Diagnosis and management of tear filmdysfunction. In : Leibowitz HM, Waring GO III, editors. Corneal disorders ,clinical diagnosis and

management . 2nd ed. Philadelphia : Saunders, 1998 : 482-500.

23. Lopez FM, Pfliugfelder SC. Dry Eye: In: Krachmer JH, Mannis MJ, Holland EJ,editors. Cornea and external disease, clinical diagnosis and management. Missouri, United States of America: Mosby year book , Inc, 1997 : 663- 686(volume 2)
24. Nichols KK , Nichols JJ, Mitchell GL . The lack of association between signs andsymptoms in patients with dry eye disease. Cornea 2004;23: 762-770.
25. Nichols KK, Mitchell GL , Zadnik K. Performance and Repeatability of the NEI- VFQ-25 in patients with dry eye. Cornea 2002;21:578-583.
26. Schiffman RM, Christianson MD , Jacobson G, Hirsch JD, Reis BL, Reliability and validity of ocular surface disease index. Arch Ophthalmol 2000;118(5):615-621.
27. Bron AJ. Diagnosis of dry eyes . Survey of Ophthalmology 2001 Mar ; 45 suppl 2: 221-226.
28. Elaison JA, Maurice DM . Staining of the conjunctiva and tear film. British Journal of Ophthalmology 1990; 74 : 519-522.
29. Natadisastra G, Wittpenn JR , West KP , Muhilal RD , Sommer A. Impression Cytology for detection of Vitamin A deficiency. Arch Opthalmal 1987 ; 105: 1224-1228.
30. Tananuvat N, Lertprasertsuk N, Mahanupap P, Noppanakong P. Role of impression cytology in diagnosis of ocular surface neoplasia. Cornea 2008; 27(3):269-274.
31. Reddy M, Reddy RP, Reddy SC. Conjunctival impression cytology in dryeye states . Ind J Opthalmol 1991;39(1):22-24.
32. Meena MKB, Khuteta A, Saxena H. Nylon paper : An alternative to celluloseacetate paper for use in conjunctival impression cytology . Br J Opthalmol 2005;1223-1224.
33. Lamberts DW. Clinical diseases of the tear film. In : Smolin J, Thoft RA, editors. The Cornea , Scientific foundations & clinical practice. 3ed edition . Little, Brown and company ; 1994 p.457-476.

34. G Abha, D Reza. Keratoconjunctivitis sicca : clinical aspects . In : SmolinG, Thoft RA, editors. The Cornea Scientific Foundations and clinical practice. Philadelphia . Lippincott Williams & Wilkins ; 616-622.
35. Erdelyi B, Kraak R, Zhivov A, et al. In vivo confocal laser scanning microscopy of the cornea in dry eye. Graefes Arch Clin Exp Ophthalmol 2007; 245(1): 39–44.
36. Kanellopoulos AJ, Asimellis G. In vivo 3-dimensional corneal epithelial thickness mapping as an indicator of dry eye: preliminary clinical assessment. *Am J Ophthalmol*. 2014;157(1):63-68.e2.
37. Cui X, Hong J, Wang F, et al. Assessment of corneal epithelial thickness in dry eye patients. *Optom Vis Sci*. 2014;91(12):1446-1454.
38. Qingfeng L, Hong L, Hanruo L, et al. Ocular surface epithelial thickness evaluation in dry eye patients' clinical correlations. J Ophthalmol 2016; 2016: 1628469.
39. Hashmani N, Hashmani S, Saad CM. Wide corneal epithelial mapping using an optical coherence tomography. Investigative ophthalmology & visual science. 2018 Mar 1;59(3):1652-8.
40. Ma JX, Wang L, Weikert MP, de Oca IM, Koch DD. Evaluation of the repeatability and reproducibility of corneal epithelial thickness mapping for a 9-mm zone using optical coherence tomography. Cornea. 2019 Jan 1;38(1):67-73.
41. Sella R, Zangwill LM, Weinreb RN, Afshari NA. Repeatability and reproducibility of corneal epithelial thickness mapping with spectral-domain optical coherence tomography in normal and diseased cornea eyes. American Journal of Ophthalmology. 2019 Jan 1;197:88-97.
42. Abou Shousha M, Wang J, Kontadakis G, et al. Corneal epithelial thickness profile in dry-eye disease. *Eye (Lond)*. 2020;34(5):915-922. doi:10.1038/s41433-019-0592-y
43. Rattan, Suzan & Anwar, Didar. (2020). Comparison of corneal epithelial thickness profile in dry eye patients, keratoconus suspect, and healthy eyes. European Journal of Ophthalmology.

30. 112067212095203. 10.1177/1120672120952034.

44. Yusufoğlu E, Kobat S and Keser S. (2022). Evaluation of central corneal epithelial thickness with anterior segment OCT in patients with type 2 diabetes mellitus. International Ophthalmology. 10.1007/s10792-022-02384-5. pithelium following surgical procedures, especially those applied to the cornea.

45. Pflugfelder SC, Tseng SC, Sanabria O, Kell H, Garcia CG, Felix C, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. Cornea. 1998;17:38–56.

46. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. Cornea. 2004;23:762–70.

47. Kanellopoulos AJ, Asimellis G. In vivo three-dimensional corneal epithelium imaging in normal eyes by anterior segment optical coherence tomography: a clinical reference study. Cornea. 2013;32(11):1493–1498.

48. Wirbelauer C, Pham DT. Monitoring corneal structures with slit lamp-adapted optical coherence tomography in laser in situ Keratomileusis. J Cataract Refract Surg 2004;30:1851-60.

49. Feng Y, Simpson TL. Comparison of human central cornea and limbus in Vigo using optical coherence tomography. Optom Vis Sci 2005;82:416-9.

50. Wang Q, Lim L, Lim SWY, Htoon HM. Comparison of corneal epithelial and stromal thickness between keratoconic and normal eyes in an Asian Population. Ophthalmic Res 2019;62:134-40.

51. El Sanharawi M, Sandali O, Borderie V, Laroche L. In Vivo 3-dimensional corneal epithelial thickness mapping as an indicator of dry eye: preliminary clinical assessment. *Am J Ophthalmol*. 2014;157(5):1116. doi:10.1016/j.ajo.2014.01.029.

52. Fabiani C., Barabino S., Rashid S., Dana M. R. Corneal epithelial proliferation and thickness

in a mouse model of dry eye. *Experimental Eye Research.* 2009;89(2):166–171.

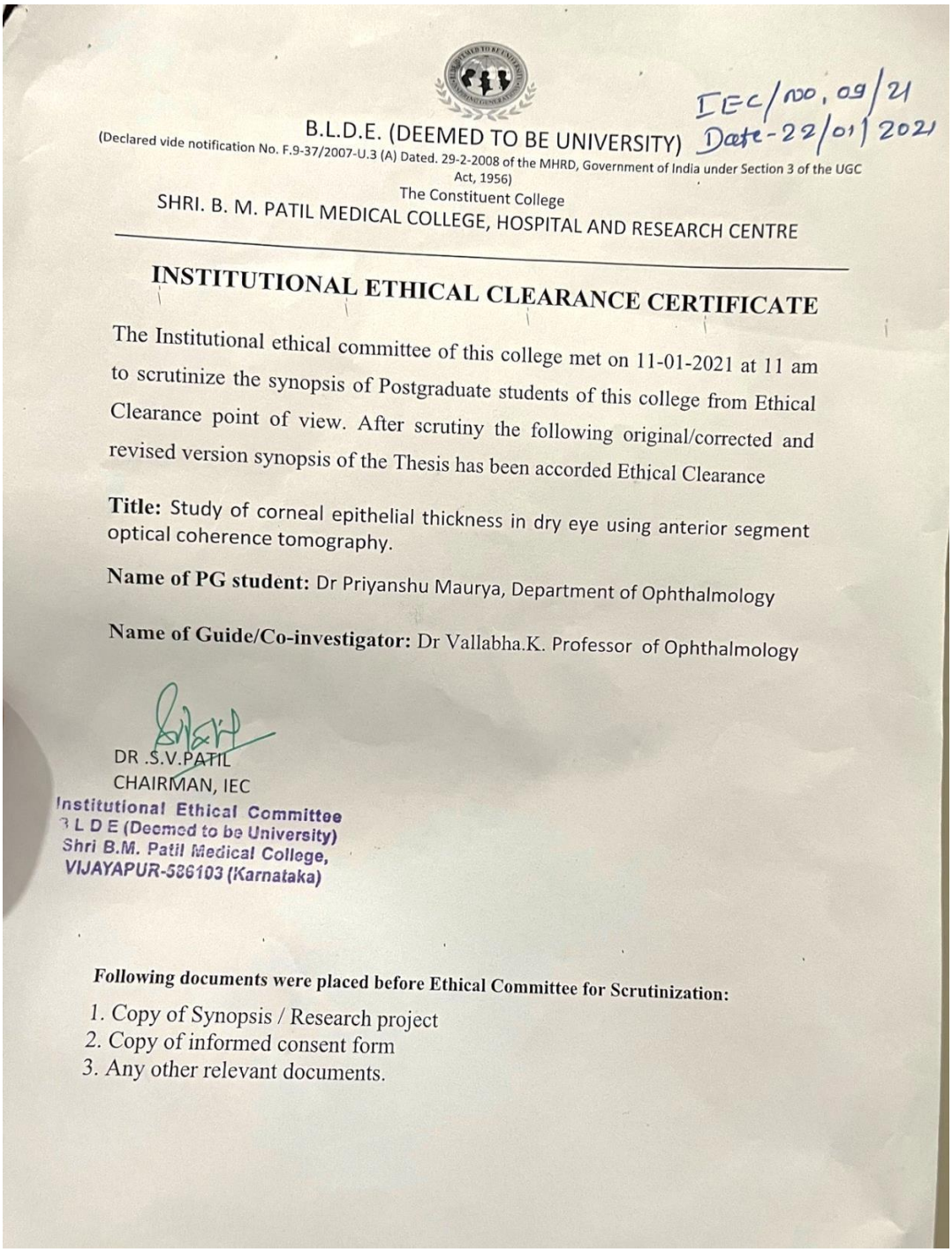
53. Chen W., Li Z., Hu J., et al. Corneal alternations induced by topical application of benzalkonium chloride in rabbit. *PLoS ONE.* 2011;6(10).

54. Villani E., Galimberti D., Viola F., Mapelli C., Ratiglia R. The cornea in Sjögren's syndrome: an in vivo confocal study. *Investigative Ophthalmology and Visual Science.* 2007;48(5):2017–2022.

55. Tuominen I. S. J., Konttinen Y. T., Vesaluoma M. H., Moilanen J. A. O., Helintö M., Tervo T. M. T. Corneal innervation and morphology in primary Sjögren's syndrome. *Investigative Ophthalmology and Visual Science.* 2003;44(6):2545–2549.

ANNEXURES

ETHICAL CLEARANCE CERTIFICATE



B.L.D.E. (DEEMED TO BE UNIVERSITY)

IEC/100.09/21
Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Study of corneal epithelial thickness in dry eye using anterior segment optical coherence tomography.

Name of PG student: Dr Priyanshu Maurya, Department of Ophthalmology

Name of Guide/Co-investigator: Dr Vallabha.K. Professor of Ophthalmology

DR .S.V.PATIL
CHAIRMAN, IEC

Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

STUDY SUBJECT CONSENT FORM

I confirm that Dr. Priyanshu Maurya has explained to me the purpose of research, the study procedure and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore, I agree to give consent to participate as a subject in this research project.

_____ _____

(participant) (date)

_____ _____

(witness to signature) (date)

RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomforts during the examination or during the treatment. The procedures of this study are not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation will help in the early diagnosis of dry eye using AS-OCT. I understand and accept the risks, benefits and costs involved. I willingly give consent to take part in the study.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs will be used only with special written permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more questions about the study to Dr. Vallabha K. in the Department of Ophthalmology who will be available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr. Priyanshu Maurya may terminate my participation in the study after she has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in the study, if such injury were reported promptly, the appropriate treatment would be available to me. But no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

_____ – _____

(participant) (date)

I have explained to_____the purpose of the research, the procedures required and the possible risks to the best of my ability.

_____ _____

Dr. Priyanshu Maurya Date (Investigator)

PROFORMA FOR CASE TAKING

DEPARTMENT OF OPHTHALMOLOGY

B.L.D.E U'S SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL

& RESEARCH CENTRE,

VIJAYAPURA-586103

Case No : OPD/IPD No: Date :

Name: Age: Sex:

Occupation: Address :

Economic status:

Time of Presentation:

Chief complains:

USE OF GLASSES
H/O OCULAR SURGERY
H/O OCULAR MEDICATION
USE OF CONTACT LENS
H/O OCULAR PROBLEMS
SYSTEMIC HISTORY

RE **LE**

VISUAL ACUITY

DISTANT VISION
PINHOLE VISION
NEAR VISION
INTRAOCULAR PRESSURE (By NCT)

OCULAR EXAMINATION	**RE**	**LE**
EXTERNAL APPEARANCE		
OCULAR MOTILITY		
EYELIDS		
CONJUNCTIVA		
CORNEA		
AC		
IRIS		
PUPIL		
LENS		
FUNDUS:- Media- Disc- Blood vessel- Background- Macula-		
OSDI		
Schirmer I test (mm)		
TBUT (seconds)		

EPITHELIAL THICKNESS	**OD**	**OS**
0-2 MM		

2-4 MM		
5-7 MM		
7-9 MM		

AS OCT

COLOR PLATES

Have you experienced any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . .	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9 (B)

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned? . . .	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

Fig 2a: OSDI scoring

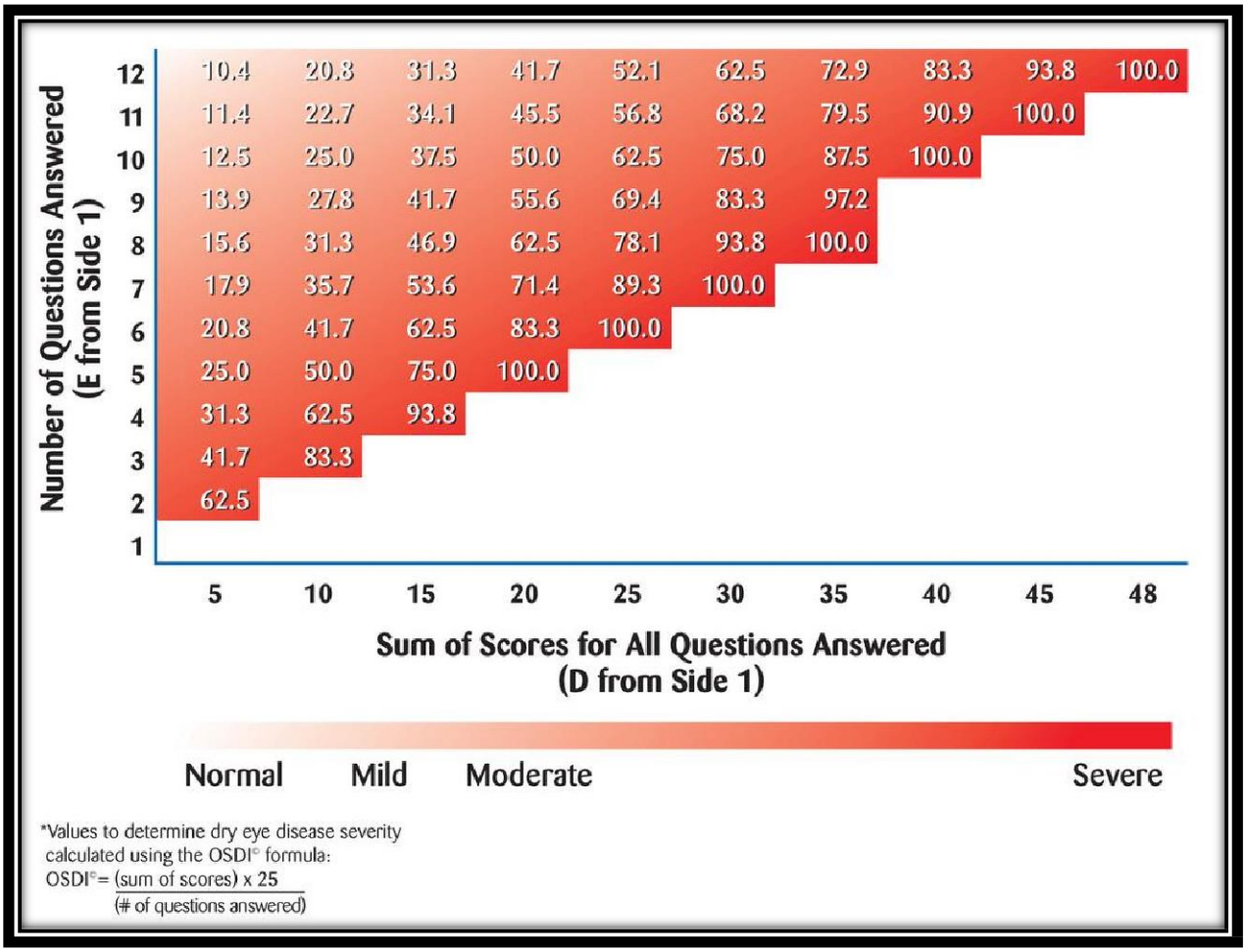


Fig 2b: OSDI scoring

Fig 3: AS-OCT in patient with dry eye

Fig 4: Fluorescein and Schirmer Test Strips

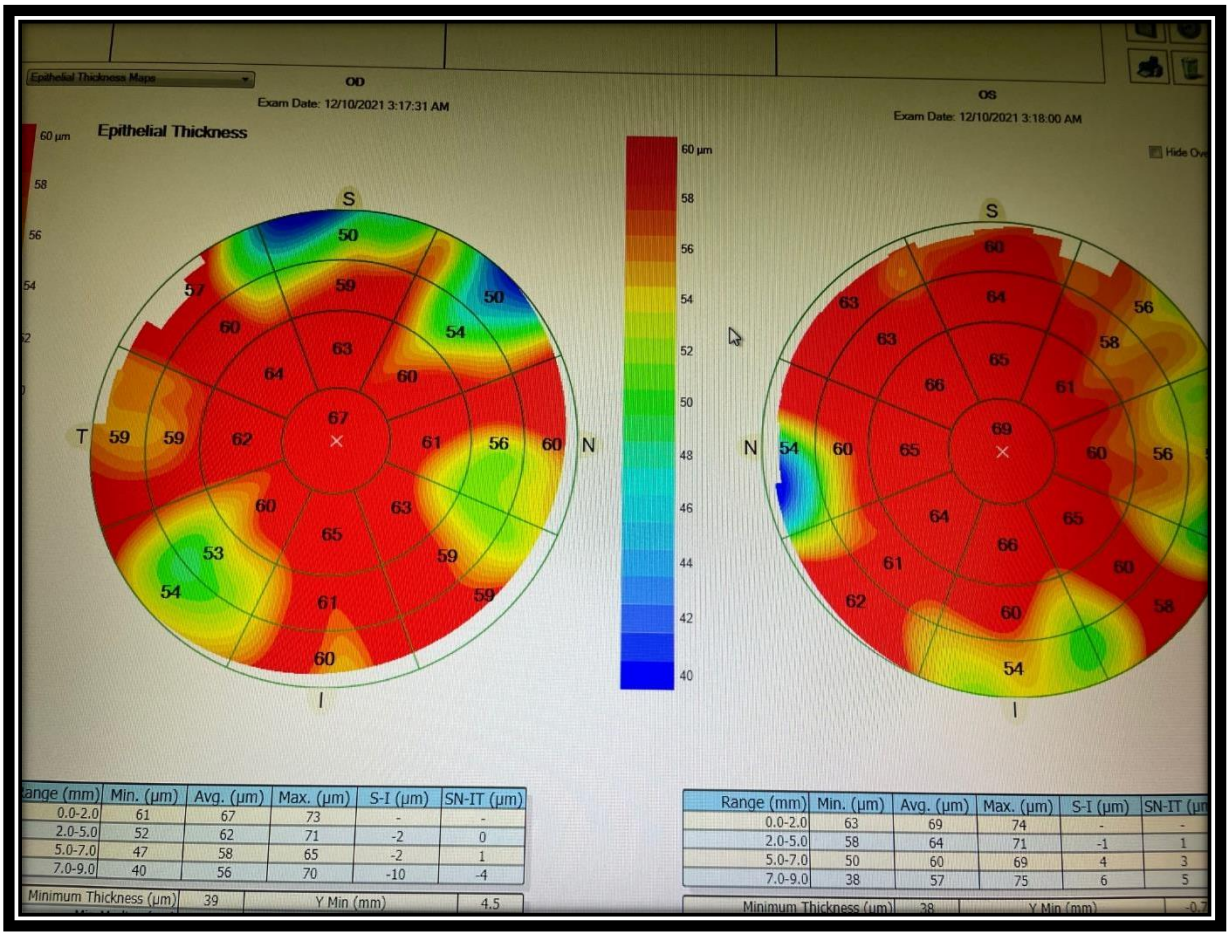


Fig 5- Corneal Epithelial thickness in dry eye patient using AS-OCT

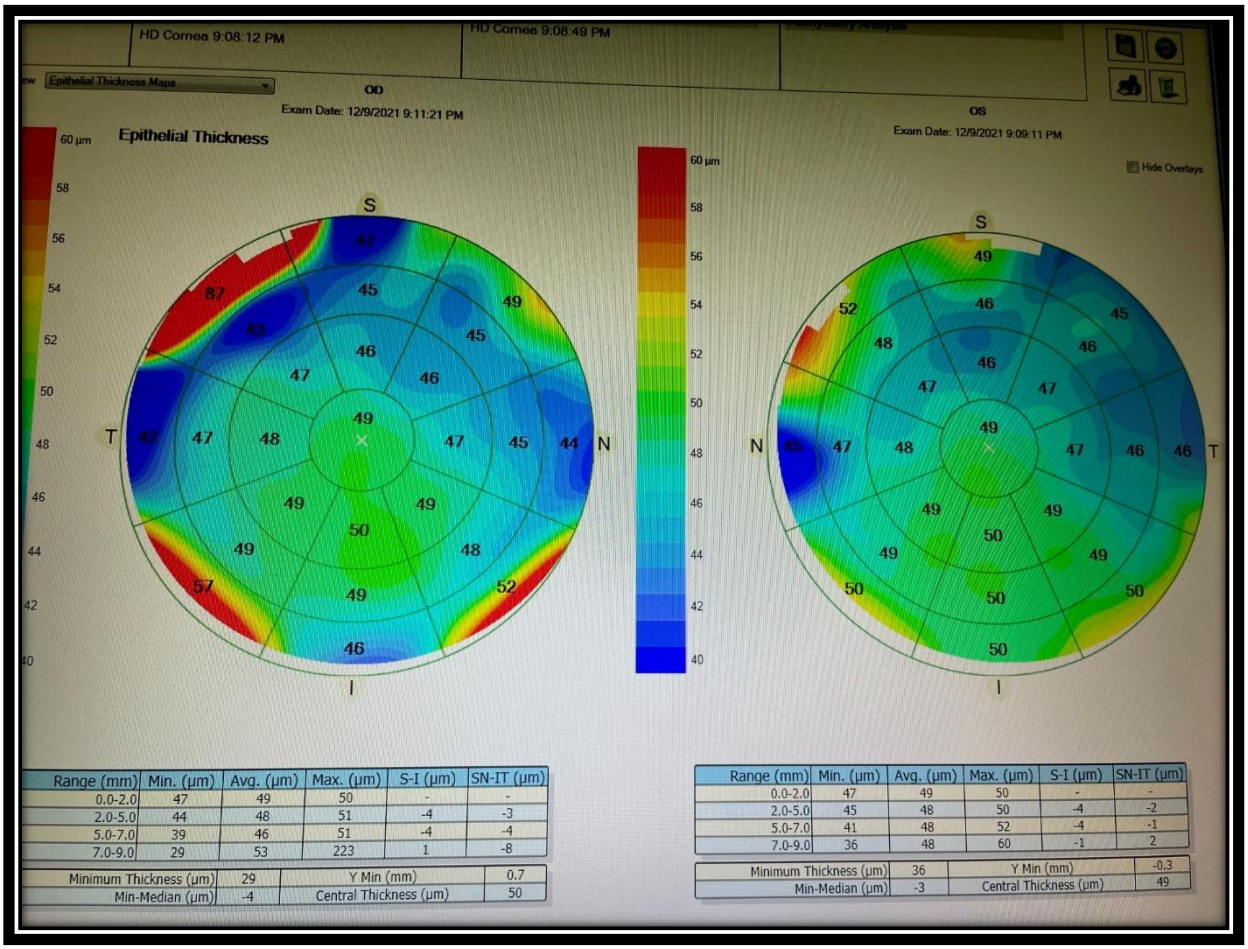


Fig 6- Corneal Epithelial thickness in control group using AS-OCT

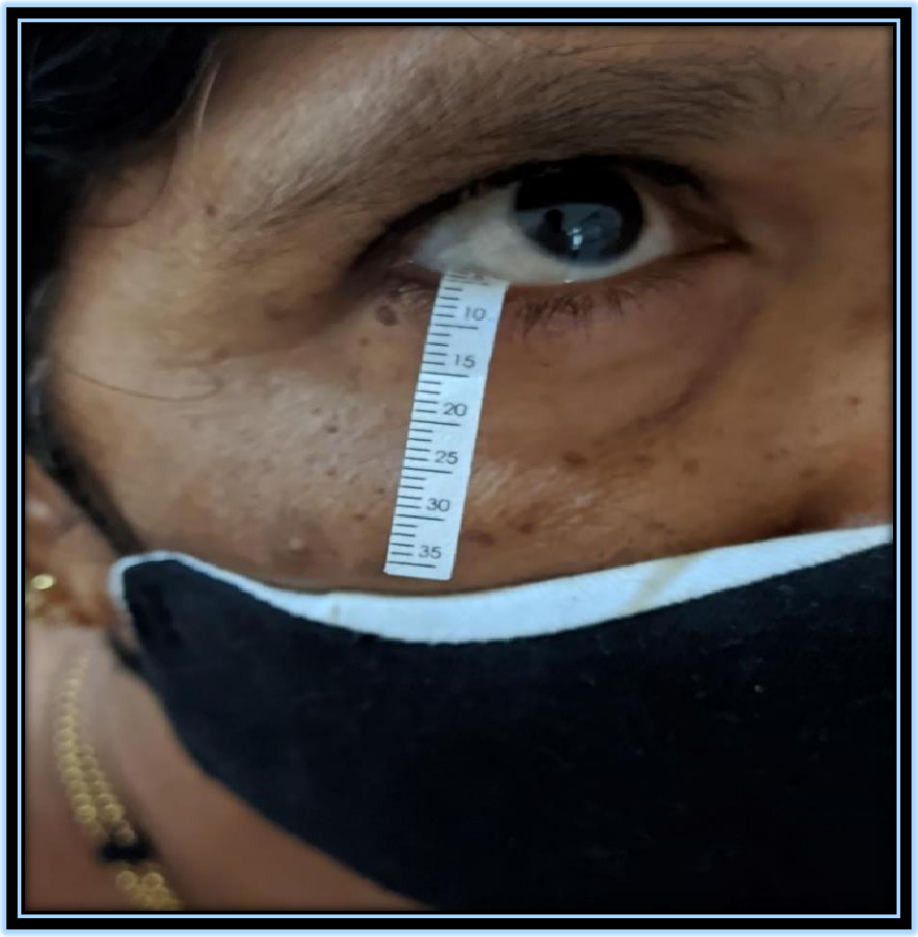


Fig 7- Schirmer Test

KEY TO MASTER CHART

C	CASE
CO	CONTROL
M	MALE
F	FEMALE
A	AGE
ST	SCHIRMER TEST
T	TBUT
RE	RIGHT EYE
LE	LEFT EYE
AV	AVERAGE
CZ	CENTRAL ZONE
Z1	ZONE 1
Z2	ZONE 2
Z3	ZONE 3

MASTER CHART

	Grou	Ger	age	OSD	STRE	STLE	STAV	TRE	TLE	TAV	CZRE	Z1RE	Z2RE	Z3RE	CZLE	Z1LE	Z2LE	Z3LE	CZAV	Z1AV	Z2AV	Z3AV
1																						
2	C	M	21	20	4	3	3.5	3	4	3.5	55	57	56	59	54	58	57	60	54.5	57.5	56.5	59.5
3	C	M	45	24	8	8	8	4	4	4	66	58	64	56	61	59	63	56	63.5	58.5	63.5	56
4	C	F	35	23	6	5	5.5	4	3	3.5	63	53	54	66	53	51	49	59	58	52	51.5	62.5
5	C	M	54	21	3	4	3.5	5	5	5	52	55	49	58	50	55	51	61	51	55	50	59.5
6	C	F	63	29	2	3	2.5	4	4	4	60	57	56	59	56	53	49	49	58	55	52.5	54
7	C	F	35	23	4	4	4	5	4	4.5	56	49	44	57	54	50	60	56	55	49.5	52	56.5
8	C	M	25	34	3	3	3	4	4	4	51	52	54	67	52	54	55	64	51.5	53	54.5	65.5
9	C	F	57	21	3	5	4	4	3	3.5	50	60	57	55	51	59	44	57	50.5	59.5	50.5	56
10	C	M	25	34	3	4	3.5	5	5	5	52	48	50	68	47	51	47	59	49.5	49.5	48.5	63.5
11	C	M	46	24	6	3	4.5	4	4	4	53	60	48	53	53	53	57	60	53	56.5	52.5	56.5
12	C	M	25	36	3	3	3	3	4	3.5	50	55	52	52	48	56	68	56	49	55.5	60	54
13	C	M	46	27	6	5	5.5	4	4	4	56	53	51	55	49	49	49	66	52.5	51	50	60.5
14	C	F	37	29	4	5	4.5	4	2	3	61	58	54	61	60	56	51	58	60.5	57	52.5	59.5
15	C	F	57	37	4	3	3.5	3	5	4	56	54	60	58	52	52	54	59	54	53	57	58.5
16	C	F	68	32	4	3	3.5	4	4	4	53	59	55	51	53	60	49	57	53	59.5	52	54
17	C	M	47	36	3	4	3.5	5	6	5.5	62	51	44	59	58	48	47	67	60	49.5	45.5	63
18	C	M	70	40	4	5	4.5	4	2	3	59	52	47	63	55	60	44	55	57	56	45.5	59
19	C	F	49	25	4	3	3.5	3	2	2.5	56	60	54	56	56	54	54	68	56	57	54	62
20	C	F	31	35	3	4	3.5	3	4	3.5	57	63	51	54	53	56	57	61	55	59.5	54	57.5
21	C	M	48	34	3	4	3.5	5	5	5	64	56	53	58	57	51	50	58	60.5	53.5	51.5	58
22	C	M	59	24	6	5	5.5	4	3	3.5	66	61	50	55	52	49	57	51	59	55	53.5	53
23	C	M	63	37	2	3	2.5	5	4	4.5	61	58	55	50	57	56	50	55	59	57	52.5	52.5
24	C	M	46	27	4	3	3.5	4	5	4.5	55	54	51	58	59	54	48	63	57	54	49.5	60.5
25	C	F	37	29	4	4	4	4	5	4.5	56	56	56	49	56	59	52	56	56	57.5	54	52.5
26	C	M	45	22	5	4	4.5	4	4	4	53	51	57	62	57	51	51	56	55	51	54	59
27	C	F	52	24	4	3	3.5	6	5	5.5	57	49	52	63	64	52	49	67	60.5	50.5	50.5	65
28	C	M	27	36	3	2	2.5	4	4	4	52	56	50	67	50	60	65	67	51	58	57.5	67
29	C	M	21	35	4	3	3.5	3	4	3.5	60	56	51	58	52	54	55	64	56	55	53	61
30	C	M	45	24	8	8	8	4	4	4	52	52	54	59	51	59	44	57	51.5	55.5	49	58
31	C	F	22	23	6	5	5.5	4	3	3.5	53	60	49	57	47	51	47	59	50	55.5	48	58
32	C	M	54	30	3	4	3.5	5	5	5	58	48	47	67	53	53	57	60	55.5	50.5	52	63.5
33	C	F	63	39	2	3	2.5	4	4	4	55	60	44	55	48	56	68	56	51.5	58	56	55.5
34	C	F	37	23	4	4	4	5	4	4.5	56	54	54	68	49	49	49	66	52.5	51.5	51.5	67
35	C	M	25	44	3	3	3	4	4	4	53	56	57	61	60	56	51	58	56.5	56	54	59.5
36	C	F	68	35	3	5	4	4	3	3.5	57	51	50	58	52	52	54	59	54.5	51.5	52	58.5
37	C	M	32	36	3	4	3.5	5	5	5	52	49	57	51	53	60	49	57	52.5	54.5	53	54
38	C	M	46	24	6	3	4.5	4	4	4	57	56	50	55	58	48	47	67	57.5	52	48.5	61
39	C	F	57	35	3	5	4	4	3	3.5	56	60	54	56	56	60	54	56	56	60	54	56
40	C	M	25	34	3	4	3.5	5	5	5	57	63	51	54	57	63	51	54	57	63	51	54
41	C	M	46	24	6	3	4.5	4	4	4	64	56	53	58	64	56	53	58	64	56	53	58
42	C	M	25	36	3	3	3	3	4	3.5	66	61	50	55	66	61	50	55	66	61	50	55
43	C	M	46	27	6	5	5.5	4	4	4	61	58	55	50	61	58	55	50	61	58	55	50
44	C	F	37	29	4	5	4.5	4	2	3	55	54	51	58	55	54	51	58	55	54	51	58
45	C	M	63	37	2	3	2.5	5	4	4.5	57	63	51	54	58	48	47	67	57.5	55.5	49	60.5
46	C	M	46	27	4	3	3.5	4	4	4	64	56	53	58	56	60	54	56	60	58	53.5	57
47	C	F	37	29	4	4	4	4	3	3.5	66	61	50	55	57	65	54	60	61.5	63	52	57.5
48	C	M	45	22	5	4	4.5	5	5	5	61	58	55	50	64	56	53	59	62.5	57	54	54.5
49	C	F	52	24	4	3	3.5	4	4	4	55	54	54	59	66	61	53	55	60.5	57.5	53.5	57
50	C	M	27	36	3	2	2.5	5	5	5	60	44	55	48	56	68	56	67	58	56	55.5	57.5
51	C	M	27	36	3	2	2.5	4	4	4	56	56	50	67	50	60	65	67	53	58	57.5	67
52	C	M	45	24	8	8	8	4	4	4	52	52	54	59	51	59	44	57	51.5	55.5	49	58
53	C	F	22	23	6	5	5.5	4	3	3.5	54	61	50	57	56	60	47	59	55	60.5	48.5	58
54	Co	M	35	19	11	12	11.5	14	15	14.5	50	45	48	46	51	51	49	46	50.5	48	48.5	46
55	Co	F	54	16	10	10	10	16	16	16	45	48	46	50	45	50	50	47	45	49	48	48.5
56	Co	F	63	14	12	11	11.5	18	16	17	52	45	51	48	47	47	47	52	49.5	46	49	50
57	Co	M	35	10	10	10	10	20	20	20	50	52	48	46	48	49	48	51	49	50.5	48	48.5
58	Co	M	25	17	11	11	11	22	20	21	49	53	51	50	39	44	46	51	44	48.5	48.5	50.5
59	Co	M	57	12	12	11	11.5	11	10	10.5	46	49	46	49	48	46	47	52	47	47.5	46.5	50.5
60	Co	F	25	9	10	10	10	12	11	11.5	51	50	47	51	51	49	45	45	51	49.5	46	48
61	Co	F	53	11	13	12	12.5	14	13	13.5	47	47	52	48	44	45	51	47	45.5	46	51.5	47.5
62	Co	M	67	10	10	10	10	16	15	15.5	50	48	51	51	46	50	49	51	48	49	50	51
63	Co	F	48	18	11	15	13	15	15	15	51	46	51	45	50	39	45	51	50.5	42.5	48	48

64	Co	M	33	13	12	14	13	18	17	17.5	51	47	52	52	46	45	48	49	48.5	46	50	50.5
65	Co	F	46	14	10	11	10.5	21	20	20.5	50	45	45	53	46	50	48	48	48	47.5	46.5	50.5
66	Co	F	19	5	15	14	14.5	24	22	23	47	51	47	49	47	51	47	51	47	51	47	50
67	Co	M	31	8	14	14	14	15	16	15.5	46	49	51	50	49	50	51	44	47.5	49.5	51	47
68	Co	F	27	6	15	13	14	16	18	17	49	45	51	47	44	45	50	46	46.5	45	50.5	46.5
69	Co	M	29	16	13	13	13	12	11	11.5	51	53	48	48	46	51	48	46	48.5	52	48	47
70	Co	F	55	8	12	11	11.5	13	14	13.5	49	51	51	51	49	50	46	51	49	50.5	48.5	51
71	Co	F	47	15	10	11	10.5	16	15	15.5	51	48	51	53	45	49	50	46	48	48.5	50.5	49.5
72	Co	M	39	17	11	12	11.5	19	18	18.5	50	47	48	47	50	46	50	49	50	46.5	49	48
73	Co	M	51	10	12	12	12	21	20	20.5	48	52	48	48	49	51	51	53	48.5	51.5	49.5	50.5
74	Co	M	48	11	10	11	10.5	18	19	18.5	46	51	47	51	48	47	48	47	47	49	47.5	49
75	Co	F	60	8	13	12	12.5	20	20	20	50	45	51	45	46	51	48	48	48	48	49.5	46.5
76	Co	F	34	16	12	12	12	17	16	16.5	49	47	44	51	49	53	47	39	49	50	45.5	45
77	Co	M	46	12	11	12	11.5	15	14	14.5	51	46	46	51	51	46	51	45	51	46	48.5	48
78	Co	M	25	6	15	14	14.5	13	12	12.5	48	51	50	48	45	50	53	45	46.5	50.5	51.5	46.5
79	Co	F	58	10	13	13	13	18	19	18.5	51	46	48	46	51	51	49	51	51	48.5	48.5	48.5
80	Co	F	28	8	14	14	14	23	21	22	45	49	46	47	52	46	50	51	48.5	47.5	48	49
81	Co	M	32	11	13	13	13	16	17	16.5	50	53	50	45	45	45	47	48	47.5	49	48.5	46.5
82	Co	M	26	17	11	11	11	19	18	18.5	48	47	50	51	47	46	48	46	47.5	46.5	49	48.5
83	Co	F	50	18	10	11	10.5	22	21	21.5	48	48	51	49	51	51	46	48	49.5	49.5	48.5	48.5
84	Co	F	63	18	10	11	10.5	11	12	11.5	47	39	48	53	51	46	47	51	49	42.5	47.5	52
85	Co	M	50	11	11	13	12	21	22	21.5	51	45	47	50	48	49	45	51	49.5	47	46	50.5
86	Co	F	22	17	12	13	12.5	18	17	17.5	44	51	52	51	50	53	51	48	47	52	51.5	49.5
87	Co	M	68	19	10	11	10.5	14	13	13.5	49	45	51	46	51	47	49	48	50	46	50	47
88	Co	M	34	10	12	12	12	20	19	19.5	50	51	45	47	48	48	46	47	49	49.5	45.5	47
89	Co	M	53	13	11	11	11	19	18	18.5	50	49	48	45	48	39	50	48	49	44	49	46.5
90	Co	M	27	8	15	14	14.5	15	16	15.5	51	46	45	51	47	45	51	48	49	45.5	48	49.5
91	Co	F	29	11	14	13	13.5	18	16	17	50	47	46	45	51	50	48	47	50.5	48.5	47	46
92	Co	M	32	13	13	12	12.5	13	14	13.5	48	52	51	51	46	45	47	51	47	48.5	49	51
93	Co	M	38	15	12	11	11.5	12	14	13	46	51	47	48	51	51	52	44	48.5	51	49.5	46
94	Co	F	41	10	13	13	13	18	17	17.5	50	49	51	48	48	49	51	49	49	49	51	48.5
95	Co	M	53	15	10	11	10.5	16	15	15.5	50	49	49	47	51	45	48	50	50.5	47	48.5	48.5
96	Co	F	44	17	11	11	11	15	16	15.5	51	46	51	51	46	52	45	50	48.5	49	48	50.5
97	Co	M	49	12	14	13	13.5	19	18	18.5	46	50	50	50	47	53	46	49	46.5	51.5	48	49.5
98	Co	M	55	16	12	12	12	21	20	20.5	51	46	47	48	52	49	49	37	51.5	47.5	48	42.5
99	Co	F	26	7	14	13	13.5	20	18	19	48	49	49	46	51	50	45	45	49.5	49.5	47	45.5
100	Co	M	33	10	13	14	13.5	15	16	15.5	51	53	44	50	51	47	48	51	51	50	46	50.5
101	Co	F	50	16	12	13	12.5	13	14	13.5	46	50	46	50	52	48	46	45	49	49	46	47.5
102	Co	F	26	10	13	12	12.5	17	16	16.5	47	51	49	51	51	47	49	51	49	49	49	51
103	Co	M	31	13	11	11	11	20	19	19.5	52	46	45	46	49	51	51	48	50.5	48.5	48	47
104	Co	F	35	9	14	14	14	15	16	15.5	51	51	50	51	49	49	45	47	50	50	47.5	49
105	Co	M	51	16	11	13	12	13	12	12.5	51	47	39	49	45	48	50	46	48	47.5	44.5	47.5